

nature

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Not a very good reshuffle

DR Robert Press is one of the least self-assuming men you could ever expect to meet and so it is ironic that whilst his tenure of the post of Deputy Secretary (Science and Technology) in the Cabinet Office has been largely uncontroversial, his departure into retirement should be a matter of great interest and lead to wide speculation and considerable controversy. Not, we hasten to add, because Dr Press is being forced to retire for any reason. It is simply that the government is taking the opportunity of Dr Press's going to phase out any vestiges of a chief science advisory post in Whitehall. It is a peculiar quirk that this is happening just at the time that in the United States President Ford is reacquiring a science adviser after three years in which there has been no science policy office in the White House.

The announcement of the change comes in a memorandum which Lord Shepherd, the Lord Privy Seal, has just submitted to the Science Sub-Committee of the Commons Select Committee on Science and Technology. Some months ago the sub-committee discovered with some amusement that it was the Lord Privy Seal's responsibility to worry about inter-departmental co-operation and coordination on matters of science, and asked Lord Shepherd to submit a memorandum on the subject.

Much has happened in the past few years, of course, to readjust the balance of power in governmental science. Seven departments now have chief scientists to oversee a research and development budget totalling many hundred million pounds, and it was perhaps inevitable that as the science policy machinery grew in the departments the clout of the man in the centre, lacking research facilities of his own, should diminish. Now, says the Lord Privy Seal, the Cabinet Office's coordinating functions can be better fulfilled by an inter-departmental committee of chief scientists and permanent secretaries, whilst any scientific initiatives or advice to the cabinet can come from a scientist to be located within the Central Policy Review Staff (the so-called "Think Tank"). All these administrative changes may sound nit-picking and trifling, but to see what they mean in reality it is only necessary to remark that from now on the Prime Minister and Cabinet will be advised on science matters by the Head of the CPRS (Sir Kenneth Berrill) to whom a chief scientist will report.

Since scientists are as status and salary conscious as everyone else, there will be some top rank people who will not consider the job as worth their while considering.

So the first problem will be recruitment. Is it to be the director of some government laboratory, asked to give up his rural environment for the suburban sprawl and a few hundred pounds extra per year? Or is some university professor, with moderate experience in policy-making, to be invited to surrender his chair and his research? In the United States people would be tumbling over each other for such a job, firm in the knowledge that it would open up new vistas; in immobile Britain there could well be difficulties in finding the right person.

The second problem is that of access for the academic to government decision-making on science matters. Of course there are tens of thousands of scientists employed directly or indirectly by government, but science is a funny, complicated thing whose truths sometimes emerge in the most illogical ways. For government to take advantage of this it needs tentacles in all directions and particularly into the academic community. The worry is that in recent years, with the growth in the number of departmental chief scientists, too much scientific advice may be coming from within and not enough from without. The latest moves to put science into the CPRS do not bode well for a reversal of this trend.

The Lord Privy Seal also announces the formation of an Advisory Council for Applied Research and Development (ACARD). The justification for this committee of twelve or so, in which Sir James Menter will play a key role, seems to be largely to provide symmetry with the Advisory Board for the Research Councils which make recommendations on research policy in the pure sciences. ACARD will have no management functions and will simply provide advice to ministers on applied research and development in the public and private sectors. Its members, it seems, will only give it their part-time attention. There are already plenty of committees and structures devoted to applied research and development, not least the chief scientists, and it is difficult to see where ACARD has any significant new roles to play except to provide yet more committee work for the same people. Its appearance can hardly be greeted with much enthusiasm. □



A controversy reviewed

The arguments over the Aswan High Dam on the River Nile continue. **Julian Rzóśka** offers this assessment

THE controversies which rage about large projects may not be resolvable in the final analysis, but at least in the case of the Aswan High Dam in Egypt they have been far from sterile. Hardly anyone seriously disputes the advantages which the taming of a once-erratic river offers in the form of regular and numerous crops and a constant supply of electrical energy. Equally, for many, some of the disadvantages have been painfully apparent—among them the waterlogging and

salination of agricultural areas now deprived of fertile silt; the salination threat to fresh water areas in a Nile delta being eroded through a similar lack of silt; and reductions in off-shore fish populations.

The Nile has been under some form of management since Ancient Egyptian times, of course, but it was in the nineteenth and twentieth centuries, when water needs rose sharply with the emergence of the Sudan as a consumer, that the river came to be used intensively. Neither country had much rainfall, and barrages and seasonal storage dams were built; a good deal of the flood surplus had to be discharged into the Mediterranean, however, because of the lack of storage capacity.

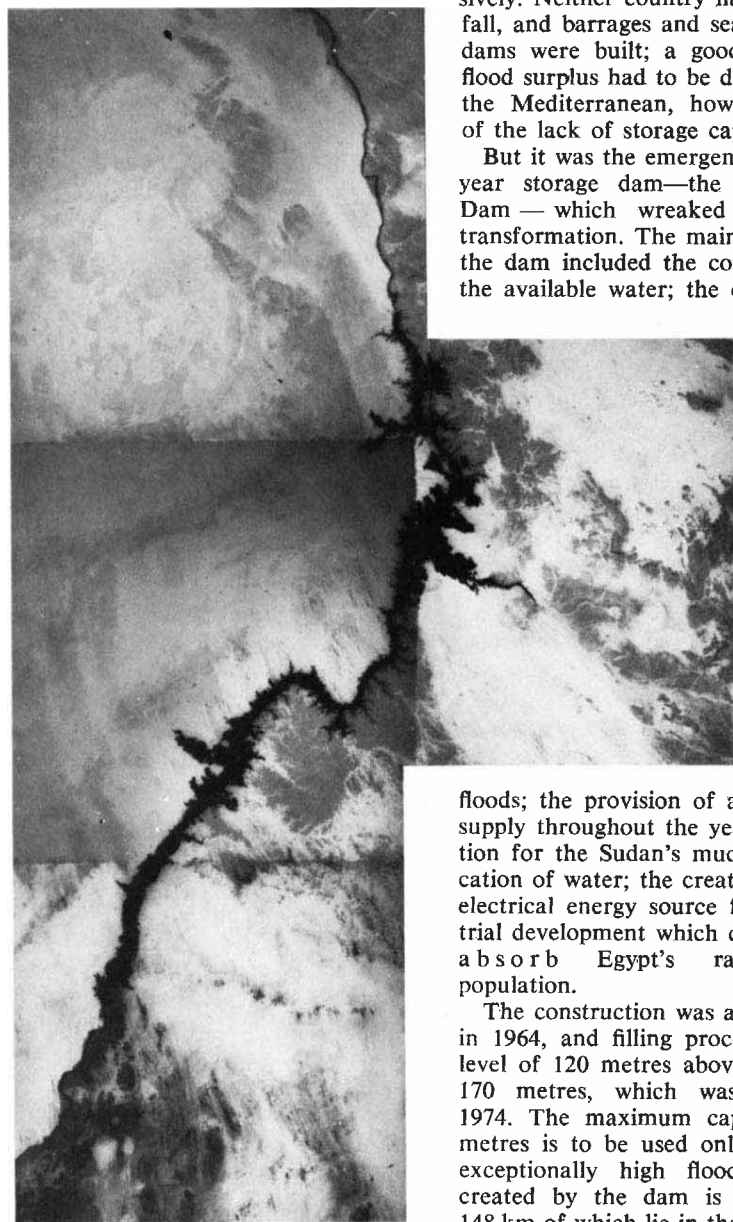
But it was the emergence of a multi-year storage dam—the Aswan High Dam—which wreaked the greatest transformation. The main objectives of the dam included the complete use of the available water; the elimination of

—oblong and relatively narrow with a dendritic outline—is typical of drowned river valleys; the shore, 9,000 km long, is bare desert.

It is not such statistics which have attracted attention, however. Indeed, few dam projects have drawn as much criticism as the Aswan High Dam, even before it was built, when the drowning of treasured antiquities was mooted. But with the dam basin now 10 years old, the Egyptians themselves have been taking stock of the main problems created by the dam, most significantly at a symposium in March at the National Research Centre in Cairo which dealt mainly with the effects downstream of the dam.

One of the main criticisms, for example, was that the dam was wasting water it was meant to save. A balance sheet, based on available hydrological documentation, shows the present situation, and is summarised in the table, presented by the chief consultant engineer Lennart Berg of Stockholm. Three important facts emerge: water available for the three areas of irrigated agriculture in Egypt and the Sudan amounts to 79,000 million cubic metres with the dam, as against 60,000 million without it; the big loss by evaporation from the lake is more than offset by the abolition of discharge into the sea; and the dam's buffering capacity allows for storage of the wide fluctuations (60,000 to 110,000 million cubic metres) of the Blue Nile floods.

The fate of the famous Nile sediments of the Blue Nile flood, which have built up the alluvial soils of Egypt, has provided the target for further objections. The chief limnologists of the project have thoroughly investigated this for the period up to 1973. Predictably, the sediments fall out where the current slackens and its "carrying capacity" is lost, and this happens in the southern part of the basin; 51% of the fallout occurs between 410 km and 345 km from the dam, and the rest in the next 60 km, almost all of it in the Sudan. Silting up of the lake basin is a long way off, however. Cautious calculations give a figure of about 300 years for the sediments to approach the dam, although this figure is regarded by some as too high. As for the loss of the sediments' fertilising effect on the soils, the delta lakes and the eastern Mediterranean, the detrimental effects are already clear in the grave reduction of the sardine fisheries in the sea and a lesser yield of the delta lakes. The new lake has admittedly created inland fisheries, which in 1973 produced an output of 10,000-13,000 tons, but the long dis-

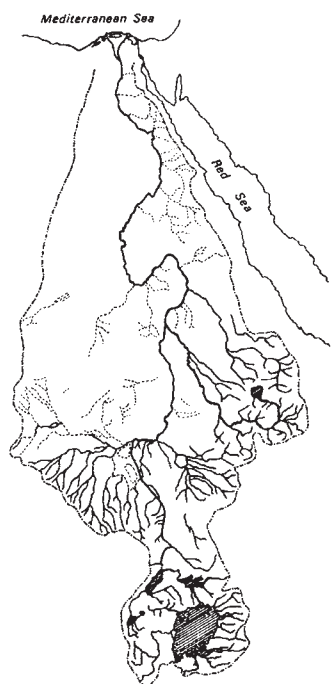


floods; the provision of an even water supply throughout the year; compensation for the Sudan's much larger allocation of water; the creation of a large electrical energy source for the industrial development which could begin to absorb Egypt's rapidly rising population.

The construction was almost finished in 1964, and filling proceeded from a level of 120 metres above sea level to 170 metres, which was reached in 1974. The maximum capacity of 183 metres is to be used only at times of exceptionally high floods. The lake created by the dam is 500 km long, 148 km of which lie in the Sudan (Lake Nubia). The area of the lake depends on how full it is, but at 180 metres above sea level it would be about 6,000 km², with a volume of 156,000 million cubic metres. The lake's shape

The Aswan High Dam basin from space: the picture, taken in 1972, encompasses about 600 km of the Nile and about 75% of the drowned area. The basin is now broader.

Illustrations from J. Rzóśka (ed.), The Nile: Biology of an Ancient River, by permission of Dr W. Junk Publishers, The Hague.



Drainage basin of the Nile system. The dotted lines represent ancient water courses

tances from Aswan make this fish more expensive than that from the sea and the lakes. The soils themselves will need artificial fertilisers.

Erosion problems have undoubtedly increased with the water head at Aswan now standing some 50 m above the previous river level and in the absence of the protective layer of sediments. The threats of river bed erosion and to bridges and river banks, all of which have been forecast, are being closely watched, and so far no serious changes have been recorded. Threats to the coast line, which existed before the dam was built, of course, are now more serious, and a team of experts of the "UNESCO-Coastal Project 1973" based in Alexandria is monitoring developments. Another effect of the loss of sediments concerns the northern dam basin itself. Critics predicted that the basin would never fill because of seepage losses into the bare river bed. Careful measurements of the amounts of water needed to fill the basin confirmed a heavy loss, but this is gradually petering out. With a rich phytoplankton already developed, biological sediment will help seal the bottom.

The perennial irrigation which now prevails raises problems of soil salination which call for more efficient drainage. These problems did not exist with basin irrigation, which was 'ecologically sound', but they are a common phenomenon in most countries relying on irrigation agriculture. The problems are most serious in the delta, which forms 60% of the cultivated land of Egypt. With a very small slope, arti-

ficial drainage by pumping will have to be increased. A change to different methods of irrigation—the sprinkler method used in Israel, for example—and the introduction of more salt-resistant crops have both been suggested, and are practised in many countries with similar irrigation problems.

Other detrimental side effects that were forecast included the spread of water-borne diseases, especially schistosomiasis (bilharzia) and malaria. Bilharzia has been in Egypt for at least 4,000 years; the last serious outbreak of malaria in Egypt occurred in 1942. Because the shores of the new lake are still bare, they do not offer opportunities for snail or insect vectors. But the weed problem has to be watched, particularly the spread of *Eichhornia crassipes*, which is rampant in the White Nile south of the Gebel Aulia dam about 1,500 km south of the new lake, and also present in the delta. Eradication of *Eichhornia* is notoriously difficult and strict monitoring will be a necessity.

The main outcry against the High Dam, however, was that no gains could justify the cultural losses caused by the drowning of a large part of Nubia and its antiquities. The narrow riverain land of Nubia, which stretches from Aswan south to about the cataract IV in the Sudan, contained remains of a series of riverain civilisations from palaeolithic onwards. About one-third of the Nubian Nile valley is now drowned. Following the salvage appeal by UNESCO, intense activity by archaeologists rescued much in a short time; Abu Simbel is only one example, and overshadows equally important results—a large documentation on the prehistory of the region, for example, and the rescue of the remains of the flourishing Christian civilisation at Faras. But much is lost.

Furthermore, the strip of alluvial land surrounded on both sides by the desert was the home of 120,000 Nubians, differing in both origin and language from both Egyptians and Sudanese. They eked out a living through scanty cultivation along the river shores, and some fishing. Many

had to seek employment in Egypt and the Sudan. But they always returned to their homeland, and were intensely attached to it. This population, straddling the political boundary between Egypt and Sudan, had to be evacuated in 1964 before the advancing floods; those from the Egyptian part went to Kom Ombo, and those from the Sudan stretch were taken far inland to Khassm el Girba on the Atbara river. This caused much bitterness and serious social consequences.

But if the value of the Aswan High Dam is to be judged, an overall assessment of Egypt's situation is necessary. The population has increased from 10 million in 1900 to 36 million in 1975. Only about 3% of the total area of 1 million km² is cultivated, although a thorough search for more cultivable land has increased the existing 29,000 km² by one-third. Perennial irrigation has increased by a factor of 1.6 the cropped area (that is, yielding more than one harvest a year). With the increased population the water share per head has actually fallen, and birth control is difficult. And the country lives almost exclusively on agriculture, because its industry is still very small. There are joint projects with the Sudan to search for new water resources—among them a canal bypassing the Upper Nile swamps with huge water losses, a very problematic project affecting the life of the Nilotic tribes of the Sudan. Other projects are under discussion.

The dam has brought Egypt a prospect for the redeployment of some of the population, as has the Roseires Dam in the Sudan. The energy potential of the Aswan High Dam is estimated at 8,000 million kW, which (other things being equal) will allow for some industrial development. The critics of the dam come from climatically and industrially far better-off countries, and must appreciate that Egyptians are aware of the dam's implications; their needs are great, and it is ultimately for them to decide how these should be met. For them, the controversies will presumably end at a time of their own choosing. □

Nile Waters during an average year
with and without the Aswan High Dam (in million m³/year)

	With	Without
Water amount available in Sudan	85,000	85,000
Allocation to Sudan	-18,500	-13,000
Evaporation losses in new lake	-11,000	—
Water passing Aswan	55,500	72,000
Irrigation of Upper Egypt	-24,300	-18,800
Recirculation gains	+ 5,300	+ 8,000
Water passing Cairo	36,500	61,200
Irrigation of Delta	-36,500	-28,200
Discharged into Mediterranean	—	-33,000
	0	0

NUCLEAR TRADE: FRANCE

Seven days in May

At the end of last month, just days after Iran had confirmed that she is to buy two nuclear reactors from France, a French consortium unexpectedly won the lucrative contract to supply two nuclear reactors to South Africa. Chris Sherwell reports

THE independence for which France has striven in her nuclear policy since the days of de Gaulle used to be confined largely to the realms of defence and the nuclear deterrent—the *force de frappe*. But times change. The last week in May was enough to confirm that, even as France assesses the balance of nuclear and conventional weapons in her defence strategy, an independent stand remains possible—in the equally sensitive field of nuclear trade, where the international consequences promise to be more widespread.

The timing of the Iranian and South African deals, as much as the content, reinforces the growing image of independence. Apart from coming ahead of important secret meetings in London last week of the expanded "Group of Seven" nuclear exporting countries, they closely followed the Canadian decision to end nuclear cooperation with India (see following story), and were announced in the face of growing US suspicion of France's controversial plans to sell nuclear reactors to Libya. The news of the South African deal also came just days after President Giscard d'Estaing's US visit, and not long after he held summit talks with leaders from the countries of Francophone Africa who gathered in Paris earlier last month.

On both occasions, it is likely that the possibility of the French consortium of Framatome, Alsthom and Spie Batignolles actually winning the \$1,000 million South African contract was mentioned, if not elaborately discussed. Anticipating criticism when the announcement finally came, the deal's defenders inside France have been quick to marshal their defences. They point to domestic employment and balance of payments benefits, but on a wider front face the problem that South Africa (like France itself but unlike either Iran or Libya) has not signed the Nuclear Non-Proliferation Treaty and has in fact recently reaffirmed her commitment to the nuclear option—facts which have enhanced the fear that South Africa might by the deal be acquiring a nuclear weapons capability.

Far from stressing that France's

nuclear trade policy does include safeguards, however, French officials maintain that no sensitive materials demanding safeguards are actually involved: in the words of one government spokesman, the contract is for the sale of "mere producers of electric current"; a spokesman for Framatome adds that plutonium produced by the two pressurised water reactors is not suitable for military purposes, and the point is also being made that South Africa has already developed the critical techniques of uranium enrichment and of fuel reprocessing anyway. The affair, says the government, should be viewed as "strictly commercial and technological".

That political factors helped crucially to decide where the main reactor contract should go is, however, openly acknowledged. The talk is of France's "reliability"; France's pragmatic foreign policy, particularly in respect of South Africa, is highlighted as a factor making the deal possible. Certainly it appears that South Africa made a judgment, probably in light of France's past arms sales to her, that while the deal would demand justification, French public opinion was unlikely to prevent it going through.

Right or not, that judgment may have been vindicated by reports on the imminence of a South African decision which stated that the leading candidate was a US-Swiss-Dutch consortium. Attention was thus helpfully drawn to the USA, where the chairman of the Africa sub-committee of the Senate Foreign Relations Committee examining aspects of the deal thought it contradicted the USA's Southern Africa policy, and to Holland, where an anguished coalition government prevaricated over the problem of its share of the credit guarantees. Certainly the proffered view of French officials is not in accord with the assessment of many observers that the French consortium won the contract by simple default when the Dutch government failed to meet its deadline.

For all that, the French press, French protestant churches, parties of the left and groups like Friends of the Earth have reacted strongly to the deal since its announcement. Reaction outside France has also grown, particularly in Africa, where the Organisation of African Unity and the African National Congress in South Africa were each quick in their condemnation. Reaction in Holland was understandably more mixed, since the impression was about that the deal had been allowed to slip from the country's grasp—some

thought by deliberate government contrivance. The Dutch government successfully survived an opposition censure motion, however.

The achievement will almost certainly contribute to the increasingly controversial debate now going on about international nuclear trade. Iran's prime minister has already denied any intention of manufacturing nuclear weapons, saying Iran is interested in nuclear power "only in order to step up electricity production rapidly and avoid wasting oil". But he has also said that Iran's 1974 agreement with France covers the whole range of nuclear technology, implying that reprocessing facilities might eventually be wanted as well. For her part France emphasises that any reprocessing facilities she provided would be controlled.

It is over the trade in both reprocessing and uranium enrichment facilities that the chief differences in nuclear export policy have arisen between France and West Germany on the one hand and the US on the other. The US would prefer a ban on such trade, while France and West Germany want trade but with safeguards and guarantees, including inspection, to prevent the misuse of the technology. As France's foreign minister puts it, for example, international life would become impossible "if one took it as a principle that international guarantees are worthless". But the Dutch reaction to the outcome of the South African deal is for many people more than enough to indicate that cut-throat competition would threaten the chances of safeguards achieving their objectives.

Already, though, the politics surrounding the latest deals has stretched far beyond the involved parties themselves and beyond questions about controls over a proliferating trade. Iran's prime minister has also had to dismiss suggestions, made in light of South Africa's existing links with Tehran and Mr Vorster's recent visit to Israel, of an emerging nuclear axis encompassing South Africa, Israel and Iran.

Similarly, there is the widespread view that the US is seriously considering what sort of support, both political and economic, it could offer South Africa in exchange for pressure from Mr Vorster on Rhodesia and movement over Namibia. Part of this support, it has been suggested, could come in the form of various types of nuclear assistance; the fact that the State Department had sanctioned the contract which has now gone to France is cited in support of this. General Electric, leader of the tripartite consortium bidding for the contract, is moreover already in on the project. It may not be

building the reactors; because the South Africans' own facilities will be on stream too late for the purposes of the project, it will be supplying them with the enriched uranium.

● In spite of mounting safety doubts and spiralling costs France reaffirmed its commitment to nuclear power by confirming in April that it is going ahead with its £500 millions Super-

phenix fast breeder reactor programme. Defending the decision, which followed earlier protests from concerned government scientists and nuclear power trades unionists, the Industry Minister, Michel d'Ornano, stressed the official view that "nuclear reactors currently present a remarkable record in the matter of safety". The French Government is aiming for an installed

nuclear capacity that will eventually meet almost all French generating demands, and if the 1,200 MW Superphenix prototype planned for the Rhone proves successful commercial breeder reactors could be under construction by 1990. Through the state-owned Electricité de France, France is a 51% partner in Superphenix with Italy and West Germany.

NUCLEAR TRADE: INDIA

An end or a beginning?

Canada recently decided to terminate nuclear cooperation with India. Our correspondent in Jullundur gives this assessment of the impact in the sub-continent.

ALTHOUGH Canada's decision was not entirely unexpected, it did come as something of a surprise because only in March this year, after nearly two years of strenuous negotiations between the two countries which had included three rounds of formal, two rounds of technical-level and several rounds of ministerial-level informal discussions, officials from the two sides initialled a detailed agreement in New Delhi which the two governments were expected to approve.

It was in this light that India responded. Speaking in the Lower House of Parliament, India's External Affairs Minister, Shri Y. B. Chavan, expressed disappointment and described the Canadian move as a "unilateral abrogation" of several provisions of the 1963 and later nuclear cooperation agreements between the two countries. He regretted that the Canadian Government had decided to "turn its back on the negotiated settlement and its contractual obligations". The Government of India, he said, was examining "the various implications" of the Canadian decision and would take "appropriate steps after this review has been completed". He made it clear, however, that there was "no ground for any suggestion that the Government of India is in any way responsible for ending Indo-Canadian nuclear cooperation".

Canada's Secretary of State for External Affairs, Mr Allan MacEachen, conveyed his government's decision to his Indian counterpart on May 18—exactly two years after India had carried out an underground nuclear explosion in the Rajasthan desert. This event is at the heart of the nuclear breach between the two otherwise very friendly countries: as Mr MacEachen put it, it was evident that Canada and India had taken profoundly different views of what should comprise peaceful applications of nuclear energy by non-

nuclear weapon states.

What effect will the Canadian decision have on India's atomic power programme? The worst that could happen would be more delays for projects that had already been delayed by an earlier Canadian ban on all nuclear shipments to India (of materials, equipment and so on) which was announced and enforced following India's explosion in 1974. The projects most affected by this ban were the second unit of the Rajasthan Atomic Power Project, RAPP-II (RAPP-I has been delivering power for quite some time now), the Madras Atomic Power Project (MAPP) and, to a lesser extent, the heavy water plant at Kota in Rajasthan, which is being built with knowhow developed at the Bhabha Atomic Research Centre (BARC) in Bombay.

For all of these projects India had placed orders with some Canadian manufacturers of special equipment, and sought alternative sources following the Canadian Government's ban. For example, critical components like reactor vessels and end-shields for MAPP reactors—originally on order from Canadian firms—have since been designed in India and are being built indigenously. The reactor vessel for MAPP-I has in fact already been delivered. Aside from a small number of specialised items which the country found it uneconomical to produce locally, most of the equipment and materials (including uranium fuel assemblies) for 200 to 250 MW CANDU reactors are being produced indigenously. RAPP-II, which after earlier delays will now be ready for commissioning in early 1977, may not possibly be able to go on steam as scheduled for want of enough heavy water. However, some heavy water is already being produced in the country at Nangal in Punjab (14 tons a year); a plant at Baroda in Gujarat (67 tons a year) was recently commissioned and is expected to start production shortly; and two more heavy water plants (Talcher, 62 tons a year, and Tuticorin, 71 tons a year) are expected to be commissioned by 1977. Thus, apart from the delays caused by the ban, any additional delays could only be

marginal as far as projects already under way are concerned.

The feeling in India is that Canada may suffer a loss of credibility by its action, and eyes are now being turned towards the US, where the government is under some pressure to default on its contractual obligation to supply India with enriched uranium for her Tarapur Atomic Power Station (TAPS), built by an American company on a turnkey basis. Shri Chavan has warned developing countries against attempts by developed countries to make participation in the technological revolution difficult: "... the Canadian thing", he told the Upper House, "is a warning in this direction", a warning to all developing countries and not only to India. India, he said, did not believe in making nuclear weapons, but it would not want to give up its right to develop nuclear energy for peaceful purposes.

The Canadian decision may yet help India move faster and with greater determination towards self-reliance and self-sufficiency in the nuclear field. Work has reportedly been going on at BARC on enrichment of uranium, for instance, and at TAPS on experiments aimed at finding ways to use plutonium in place of enriched uranium in the TAPS reactors. How much progress is being made is not known, however. Canada's action meanwhile raises questions about safeguards. When the agreement was signed between India and Canada in 1963 for setting up RAPP-I, Canada wanted India to agree to inspection by the International Atomic Energy Agency (IAEA), but India did not agree. An understanding was reached whereby Canada could inspect India's RAPP reactors and India could in turn inspect Canada's Douglas Point reactors, but when Canada later supplied heavy water for commissioning RAPP-I, it insisted that India sign a trilateral agreement involving the IAEA which put RAPP-I under joint safeguards. India is now presumably free to refuse any inspection of RAPP-I by Canada or by the IAEA, and free to use plutonium generated in the RAPP reactors any way she chooses. India has, after all, been using her own uranium in her reactors, including RAPP-I; only TAPS uses enriched uranium supplied by the USA under a contract. □

USA

Research budget setback for NSF

The Ford Administration's plans to boost spending on basic research in the United States have collided with election-year politics in the House of Representatives, and it now seems likely that support for many areas of research will continue to be eroded by inflation at least for another year. Colin Norman reports from Washington

THE House Appropriations Committee, the most powerful of the Congressional budget committees, has approved an appropriations bill for the National

Science Foundation (NSF)—the chief source of federal support for non-biomedical basic research—which would reduce the foundation's proposed research budget by nearly \$60 million. The bill would provide \$681 million for NSF's research programmes next year, a 6% cost-of-living increase instead of the 20% boost proposed by the Ford Administration.

The bill will reach the floor of the House during the last week of June, but few upward revisions of the figures should be anticipated there. The Senate will then have its say on the measure, and although it has traditionally been

more generous than the House towards NSF, it seems certain that the final, Congressionally approved, budget will fall far short of the Administration's request.

Why is Congress taking such a parsimonious attitude towards NSF? The answer is complicated but, essentially, the Appropriations Committee did not buy the Administration's arguments for increasing research support at a time of general fiscal restraint.

Dr. Guyford Stever, the director of NSF, stated the Administration's rationale most clearly when he described the NSF budget request to reporters last January. "The specific aim" of the proposed 20% boost in NSF's research support, he said, "is to

● In a report sharply critical of some of the policies of the Energy Research and Development Administration (ERDA), the Congressional Office of Technology Assessment (OTA) has concluded that "achieving energy independence by 1985 has become all but impossible". Moreover, since 37% of the oil consumed in the United States last year was imported and the proportion has now reached about 40%, OTA suggests that "even to hold import dependence to the present levels through 1958 would be a formidable achievement. The energy situation is serious and deteriorating".

The report, an analysis of ERDA's programmes and budget requests, suggests that although ERDA has made substantial progress during its first year in forging an energy research and development strategy, there are still serious deficiencies in its approach. And there are even more serious defects in the budget allocations approved by the Office of Management and Budget (OMB).

In particular, OTA argues that ERDA is paying insufficient attention to policies which could affect the energy situation in the next five years. Although ERDA's recently announced energy plan places greater emphasis on conservation, OTA points out that such efforts still account for only 3.8% of ERDA's budget. Moreover, "the conservation programme has no overriding sense of direction", OTA charges. As for solar energy, OTA suggests that the budgets proposed for solar programmes—particularly the development of heating and cooling units—are much too small.

In many instances, the fault lies not with ERDA but with OMB. ERDA officials argued, for example,

that conservation efforts should receive some \$193 million in the fiscal year which begins on October 1, but OMB allowed the agency to request only \$113 million in its budget application to Congress. Similarly, OMB sliced deeply into ERDA's proposals for solar energy before submitting the agency's budget to Congress.

OTA's analysis has already reached sympathetic ears in the Congress. Though the study was only published last week, drafts have been circulating around Capitol Hill for some time, and they have been used to brief members of key committees. Consequently, on May 25, the House Appropriations Committee approved a bill for ERDA which added \$50 million to the request for solar energy development and \$10 million to the budget for conservation efforts.

● In a decision hailed by various nuclear critics, a Federal Appeals Court in New York last week ruled that plutonium cannot be used as a reactor fuel in the United States until the federal government has completed a thorough study of safety and other issues surrounding its use. The immediate impact of the decision will be to delay the start-up of the only plant designed to reprocess commercial reactor wastes in the United States, but the chief effect could be to put off the recycling of plutonium for several years—a pause which nuclear critics are likely to put to good use.

Last November, the Nuclear Regulatory Commission (NRC), the agency responsible for regulating the nuclear industry, announced its intention to issue temporary licences to allow plutonium to be recovered from reactor wastes and recycled as a fuel, pending completion of a massive environmental impact statement on

the matter. NRC's decision would have enabled a reprocessing plant, located at Barnwell, South Carolina, to begin limited operations next year. Though NRC made clear that the temporary licences would be revoked if the environmental impact assessment indicates that plutonium recycling is undesirable, nuclear critics were concerned that commercial plutonium use, once started, would be difficult to stop.

The Appeals Court agreed. It directed NRC to complete its environmental study—now scheduled for August—and to conduct public hearings on the issue before approving any licences. The whole process is unlikely to be completed until late next year.

Plutonium recycling is important to the nuclear industry (a fact underlined by the appearance in court of 28 nuclear and electric utility companies) because it will eventually supplement dwindling supplies of uranium. It will also be vitally important for a nuclear programme based on the breeder reactor. But there is widespread concern that commercial use of plutonium will greatly increase the chances of diversion of fissile material to clandestine nuclear weapons, and there is also concern about the health hazards associated with plutonium use. Moreover, it should be noted that the United States is trying to persuade nuclear exporting countries not to transfer reprocessing plants to countries which do not already possess nuclear weapons, arguing that plutonium reprocessing at present is uneconomic. A decision by the United States to allow domestic reprocessing and plutonium recycling would be viewed abroad as hypocritical, at best.

counteract the gradual decrease of federal support for basic research, which has declined by about 23% in terms of constant dollars since 1968". Although research budgets have been going up steadily since the early 1960s, inflation has been increasing even more sharply and the purchasing power of the research dollar has consequently been shrinking.

Those trends were documented earlier this year in a study prepared for the National Science Board, NSF's policymaking council. The study, *Science Indicators 1974*, was said to have been particularly influential in persuading the White House Office of Management and Budget (OMB) to back the proposal for a large expansion of federal support for basic research. It showed that total expenditure on basic research (both public and private) increased from \$1,200 million in 1960 to about \$4,000 million in 1974, but that with inflation factored in, research support peaked in 1968 and by 1974 had fallen to the 1965 level.

In its written report on the NSF appropriations bill, however, the House Appropriations Committee didn't agree that basic research is in financial trouble, suggesting instead that "there may be problems of interpretation of the data" in the *Science Indicators* study. In particular, the committee notes that during the early 1960s, federal agencies tended to maximise their reported support of basic research but more recently, when the watchword has been "relevance", they have tended to classify research as applied whenever possible. Those factors have tended to skew the trends. Equally important, the committee argues that research budgets rose very sharply in the early 1960s, to reach a high level

of federal support, and "this base has continued for the past decade". Moreover, the committee quotes Dr Stever as describing the United States' research and development effort as "still the strongest in the world".

In short, "after carefully considering all factors relating to NSF's research support programs, the Committee feels that the Foundation should be given the budgetary resources needed to continue its current level of research support". In addition, the committee notes that since the research budgets of a few other agencies, such as the Environmental Protection Agency and the Energy Research and Development Administration, have been increased, there seems to have been a marked shift in basic research priorities. The broad policy implications of that shift need to be studied by the new White House Office of Science and Technology policy and, perhaps, by the next Administration, the committee argues.

It should also be noted here, however, that for the past couple of years, NSF has been accorded considerable adverse publicity because of its support of supposedly trivial research projects. Several Senators and Congressmen have managed to get their names into the newspapers by taking cheap shots at research grants with funny-sounding titles and, consequently, there is a strong public perception that the foundation is wasting taxpayers' money. The committee notes in its report that there have been "major concerns in management and administration of NSF programmes", but it stops short of saying out loud that it isn't politically very easy to increase NSF's budget in an election year.

As for other parts of NSF's budget, the House Appropriations Committee

was more generous. It increased, by \$9 million, the Administration's budget request for science education activities, including a \$3 million addition for courses to acquaint teachers with new school science curricula and new teaching methods. The bulk of NSF's education funds are channelled into universities and colleges, and provide a jealously guarded form of support for higher education. They therefore have considerable popular appeal, which is one reason why the committee preserved them from the axe.

Meanwhile, on May 27, the Senate passed a bill which would broaden NSF's activities and change some of the foundation's management practices. Sponsored by Senator Edward Kennedy, the bill would, among other things, increase public participation in the formulation of NSF policies by increasing the numbers of lay members on NSF committees and advisory panels. It would establish programmes to improve opportunities for members of minority groups to study science and engineering, and it would also establish a new programme of grants to enable state governments to strengthen their science policy machinery. A House-passed version of the bill is, however, much less ambitious and it is likely that some of the provisions in the Senate version will be dropped before the measure is given final Congressional approval. Some NSF officials have also expressed reservations about Kennedy's bill, arguing that it would steer the Foundation further away from its central mission of supporting basic research.

The chief worry of NSF officials, and their clients in the universities and colleges, however, is that Congress now seems likely to prolong the 10-year erosion of support for basic research.

AUSTRALIA

Back to two o'clock

Peter Pockley reports from Sydney on developments involving the institutions concerned with Australia's science policy

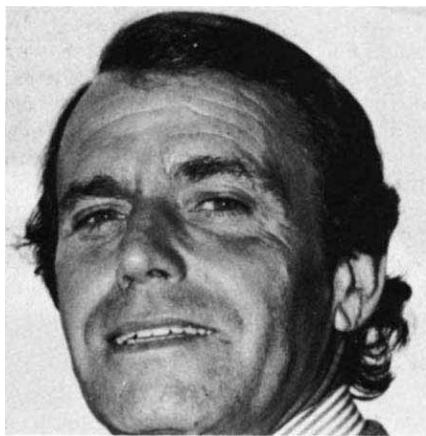
In spite of strong pressures for its abolition, the Australian Science and Technology Council (ASTEC) has survived. The Prime Minister, Mr Malcolm Fraser, has announced that the council, established last year by the Whitlam Labor government, will be reconstituted and continue for a further 12 months in an interim capacity. ASTEC, then, becomes one of the few of Labor's initiatives to survive the Fraser razor, and the scientist-politicians involved in the fight for

ASTEC can be satisfied with their efforts. Survival was necessarily their prime goal, but it appears that this has been offset by the acceptance of uncertainties about the Council's functions and influence. The real bureaucratic battle has yet to be fought and it looks likely that ASTEC, in its reconstituted form, will have no influence over the 1976-77 Budget priorities.

The science policy clock started ticking in Canberra about four years ago when the Labor Party became the first political party in the country to enunciate a science policy. Before then, the Liberal and Country Parties, through successive Ministers for Education and Science including Mr Fraser, had repeatedly set their faces against defining, or even attempting to

define, a science policy. Spurred on by leading scientists to respond to the ALP initiative, the Liberal government of Mr William McMahon appointed an 11-man Advisory Council for Science and Technology (ACST) in the last months of the government's life. The clock advanced to one o'clock.

On Labor's accession to power in December 1972, the Department of Science was formed. The clock spurted ahead to four o'clock, but dropped back to three early in 1973 when the ACST was axed and no replacement body for advice was appointed. The clock stopped dead for a year while science policy, finance and administration marked time. The visit in 1974 by the Organisation for Economic Co-operation and Development (OECD) to study Australian science policy started the clock again, and by early 1975 it had reached six o'clock with the an-



Fight on his hands: Senator James Webster, Minister for Science

nouncement that ASTEC would be born. By the year's end the hour hand had started to climb up to base with ASTEC starting to sort out its priorities and gearing up to offer effective advice. Seven o'clock had been reached with the longest hours of labour ahead before a coherent set of principles and proposals could be formulated.

Then, in December, Labor fell; time went into reverse. Mr Fraser announced the retention of ASTEC before zero was reached; the time is now two o'clock, a small but definite advance on the Liberals' last position on the formulation of science policy. Here the temptation must be resisted in trying to stretch the analogy further, lest it turn into a clock paradox.

ASTEC's Secretariat has been transferred in body and soul from the Department of Science (where the mutual relations were markedly uneasy) to the Department of the Prime Minister and Cabinet; it has the status of a bureau and will report directly to the Prime Minister. The question of statutory independence has been postponed to the end of 1976, and although not generally favoured by the Liberal's approach to controlling advisory bodies, has not been ruled out. The Minister for Science, Senator James Webster, has publicly accepted the move although it has notably downgraded his role in Cabinet and his Department's chances of survival. For example, the Advisory Group of five men set up to examine ASTEC earlier this year accepted lock, stock and barrel the terms of reference spelled out by Mr Fraser himself during the December 1975 election campaign, viz:

The Council will be the major independent advisor to Government on such matters as:

1. The development and application of science and technology to national needs and objectives.
2. New areas of science and technology which are of importance to Australia, including fields of industrially and com-

mercially oriented research and development.

3. The balance, adequacy and effectiveness of national efforts in various fields of science and technology, including defence science, and means for improving efficiency in the use of resources.

4. The relative importance of efforts in those fields of science and technology which may contribute to national economic and social development and welfare and to the advancement of scientific knowledge.

5. The effective development and utilisation of scientific and technological manpower.

The first Interim ASTEC had established a number of Task Forces. These have been formally scrapped in name only, for "ad hoc committees and working parties will have to be used for specific tasks . . . Before establishing a committee or working party the Council should obtain Prime Ministerial approval". Maintaining a fine balancing act between independence and being firmly in Mr Fraser's pocket may be helped by a provision for "early publication of Council reports . . . for comments from the scientific and the industrial community before firm decisions on policy are taken by Government".

A further period of uncertainty and delay in bringing ASTEC to a full flowering has been ensured, though, by Mr Fraser's direction that "the first task of the Council will be the preparation, by the end of 1976, of a definitive report . . . on the long-term future of ASTEC." Self-examination, again.

Six of the original 12 members of ASTEC have already been put off, with an accompanying degree of public fuss. The remaining six include the original Chairman, Dr Lewis Matheson, who has been retained in a part-time capacity. The Advisory Group has ensured its own continuing influence by successfully recommending that they be appointed en bloc to the reconstituted Council. Two of the Advisory Group (Dr Matheson and Professor Robert Street, an increasingly influential figure in Australian science politics) were already on the previous Council; the other three included two from the Australian Academy of Science. With at least five members on the new Council, the Academy has the dominant scientific voice. Remaining places on the Council are certain to be filled by people from industry and commerce.

ASTEC's retention and transfer to the Prime Minister's direct responsibility is the clearest signal yet that the Department of Science and its Minister has a major fight on its hands for survival. ASTEC is now in a position to assess the Department of Science's own priorities. The Department is listed as but one of five Federal Departments plus one Agency (CSIRO) with signi-

ficant research and development budgets which will be "invited to attend all meetings of the Council but without voting rights and without any responsibility for the Council's decisions."

Further, the Minister for Science publicly acknowledged at the ANZAAS Congress in Hobart on May 10 that his Department is under real threat of abolition. He blamed the report of the Science Task Force of the Royal Commission on Australian Government Administration for "doing a disservice to the scientific community in advocating the abolition of the Department". Senator Webster was merely reflecting the understandably critical stance of his Department's Secretary, Sir Hugh Ennor, to the draft of the Task Force's report released in January. Unrepentant, the Task Force's final report to the Royal Commission has stuck to its original recommendations that the operational branches and sections of the Department be distributed around other departments with scientific components. The Royal Commissioner, Dr H. C. Coombs, is now considered likely to accept these recommendations in his report due before the end of June.

Running parallel to the Royal Commission, which had been set up by the Labor Government, is Mr Fraser's own closed inquiry into the structure of the Australian Public Service. Headed by a former public servant, Sir Henry Bland, this committee is sending tremors throughout Canberra. If it runs true to the spirit of its founding father, who likes to be seen as a man of steel, the Bland Committee will recommend (again shortly) that various departments and agencies be amalgamated or some of their functions transferred to the six States of Australia. The Department of Science is a prime target.

In all of these hassles, the Labor Party and its science spokesman, Mr Rex Connor, have maintained a level of public disinterest which has done nothing to enhance their reputation as anything more than the initiators of science policy discussions at a political level.

Among scientists, a sense of *déjà vu* has set in. A dreary and poorly attended symposium on science policy at the ANZAAS Congress was in sharp contrast to lively symposia on the subject at the previous two Congresses held during Labor's reign. The debate has given all the appearances of degeneration into internalised rows about administrative structure (for example, ASTEC versus the Department of Science). Questions of the content of research and development and national priorities for research funds seem, for the moment, to have been put into the "too hard" basket. □

IN BRIEF

Nuclear actions

On paper, at least, the world became a safer place last month, when Japan became the 96th nation to ratify the Non-Proliferation Treaty (NPT) while the Soviet Union and the USA signed a treaty limiting the size of single nuclear explosions "for peaceful purposes" to 150 kilotons or less. The US-USSR agreement was officially hailed as a major breakthrough because of its provision for on-site inspection by either party of single explosions over 100 kilotons or multiple explosions below the upper aggregate limit of 1,500 kilotons; this, it is claimed, could clear the way for future on-site monitoring of military test blasts.

But with no satisfactory conclusion to the Strategic Arms Limitation Talks in view, and no provision for on-site inspection in the 1974 threshold agreement on military tests (which forms the major part of the newly completed treaty), liberals regard the achievement sceptically, particularly as 150 kilotons is seen as a meaninglessly high limit. Both the military and non-military treaties are expected to come

into force simultaneously following Senate ratification.

Japan's ratification of the NPT, ending a stormy six-year passage through the Diet, came as a result of strong US pressure, according to some Japanese government sources; US interests supply Japan with enriched uranium for her nuclear reactors. Japanese determination to achieve greater nuclear independence is, however, emphasised by its \$8,400 million nuclear fuel strategy aimed at securing an annual 21,700 tons of uranium oxide by 1995. Japan hopes to build up 130 million kilowatts of nuclear power in place of petroleum over the next 20 years. By 1980 the proportion of domestic enrichment should have risen from 2 to 33%.

Meanwhile, amid European plans to dump 6,700 of packaged radioactive wastes in 4,500 metres of Atlantic water next month, news broke of the first detected leak of plutonium from waste drums dumped off America over the past 20 years. Plutonium, a carcinogen threat, returns to man through the food chain.

Moves over fishing limits

The agreement reached last week between Britain and Iceland to end the six months' old Third Cod War will itself last six months, during which Britain hopes to resolve two problems largely responsible for its interim character: the question of 200-mile international limits, which the unfinished UN Law of the Sea Conference will discuss further in August; and the matter of an EEC Common Fisheries Policy, on which the Nine seek agreement regarding wider exclusive national limits inside a 200-mile EEC "pond".

Delay on both has complicated the position of Norway as well as of Iceland, and she too may operate the 200-mile limit she seeks in the absence of an EEC agreement. Britain's special difficulty as the manoeuvring amongst the Nine continues is that her reported "target" limit, in the 35-50 mile range, though higher than the current 12-mile exclusive limit, is far less than the 100 miles sought by British trawlermen. For all countries, however, the top priority remains the conservation of fish stocks.

EXPERIMENTS with human subjects are increasingly being restricted by regulations and decrees. This is probably just as well, as long as the pendulum doesn't swing too far. Vertebrates in general are now regarded with a kindly eye, unless they are considered to be edible. "The more I see of some people—any people—the better I like sea-lions". That is the cry. Experiments with *Escherichia coli* are getting a hard look. Soon we may hear that bacteria have rights, too. After all, the real difference between them and us is a disparity in the length and sequence of DNA molecules.

On the human scene, the California law-makers are pondering a bill (S-2051) saying that various categories of people cannot give "informed consent" to experiments performed on their bodies. Included under this protective umbrella are prisoners. Graduate students are not mentioned; I noted this with some nostalgia, for I am a relict of an age when we assumed risks to gain benefits. Not any more. Let the other guy take the risks. And yet . . . as Charles Best said,

So now Banting and I rolled up our sleeves. I injected him with our extract and he injected me—we had to be sure it wasn't too toxic to be tolerated by human beings.

Someone, in December, 1921, had to be the first to be injected with insulin, so that many who could not, or did

not, give "informed consent" would benefit in years to come. The first patients to receive insulin did so without talking to their lawyers about malpractice suits.

Human testing**THOMAS H. JUKES**

It was a romantic era; there were giants in those days. One was Howard Taylor Ricketts, who lifted five lice from a patient with typhus fever, and put them in his pocket in an envelope. When Ricketts opened the envelope

later, in his laboratory, there were only four lice; the other one had escaped to his body. Ricketts died of typhus fever some days later. He was not the last to die of a laboratory infection while working on typhus, before it was brought under control by yolk-sac vaccine, DDT and tetracycline antibiotics. The US Public Health Service, Hamilton, Montana laboratory published a bulletin in the 1930s that was dedicated by its authors to their colleagues who had died of Rocky Mountain spotted fever. The laboratory was conveniently near to a canyon that supplied ticks with a "hot strain" of the infective agent.

Prisoners often gave their informed consent to experiments performed on them. In some cases, their professed motivation was a desire to pay a debt that they thought they owed to society, rather than the hope of special privileges. The soldiers who volunteered to be bitten by mosquitoes that had fed on yellow-fever patients were compensated, if they survived, by sums of \$300. The widow of one of them received an annual pension of £1,500.

Most experiments with human subjects involved very little risk. I remember, as a graduate student, drinking a beaker of milk that had been liberally laced with staphylococcus toxin. Nothing happened, and I collected ten dollars. Gone are those bad old days; the lawyers and the government are looking after us.

correspondence

Selling the Sun

SIR,—Since I am part owner of what I believe to be the only completely privately built and financed house in Britain to incorporate solar central as well as water heating, I would like to present some information which could have a bearing on your editorial (May 20, page 177).

The installation was designed by Stephen Szokolay, who also designed the Milton Keynes House. Central heating is by circulation of warm water through pipes in the floor, to maintain a background warmth; water heating is by a heat exchanger in a conventional domestic tank.

In the absence of a systematic monitoring programme—it being difficult to get any financial support for what most people clearly thought was either a con-trick or the vapourings of a diseased mind—I have estimated that of the approximately 17,000 kWh annual heating demand predicted by the designer, the sun has supplied in the order of 9,000 kWh, possibly more, but certainly not much less, during the first year of operation.

The house is insulated to above-average standards, and the underfloor pipe system ensures that in summer months on very hot days, excess hot water can be pumped through the floor, which thus acts both as heat sink and heat store. If the heat decay curve for the floor slab is flat enough, some residue of surplus summer heat could possibly be retained into autumn.

Electrical immersion heaters and a wood stove are used as back-up heating for sunless days. During last winter there were periods of up to 2 weeks when the solar input could only be described as negligible. In the absence of a completely new style of house design, I cannot see how the need for a back-up system is to be avoided.

Which brings us to costs. First, energy costs: my rather crude calculations lead me to conclude that the energy cost of materials over and above what would be required for a conventional fossil-fuel piped heating system (that is, collector panels and glazing, storage tank, anti-freeze, piping, pumps, switches) is in the order of 20–25,000 kWh. The system needs to run trouble-free for at least five or six years in order to pay for itself in energy terms.

Second, raw material costs: several hundred extra kg of assorted metals

and a similar amount of glass may seem rather a high price to pay for “free heat”. There is no way in which the sun can pay off the costs. Third, financial costs: because the suppliers of components were extremely generous, the final cost of the installation, at about £1,200 (at 1974–75 prices) can be described as a bargain. We must clearly wait rather more than 5–6 years for the sun to pay that off for us.

I present these points for interested readers to ponder. But as one who hates waste, I must say that when the sun comes out and I hear the switches go on, I get a warm glow.

J. B. WRIGHT

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Birds on the Chagos Bank

SIR,—We share the concern expressed by Messrs. Hiron, Bellamy and Shepherd (*Nature*, 260, 387; 1976) for conservation in the Chagos archipelago, but we should like to comment on their proposal to exterminate the rats there. The fourteen islets on the western edge of the Chagos bank are only a fraction of over sixty in the whole Chagos archipelago, and over six hundred in the whole Indian Ocean, considered worthy of assessment for conservation by the International Union for the Conservation of Nature (Elliott, H.F.I., *J. mar. biol. Ass. India*, 14, 578–608; 1974). Many of these are of more scientific importance and more seriously threatened. Before scarce resources are devoted to an attempt to eliminate rats from the two largest of the western Chagos islands—something which is likely to be difficult and costly and has rarely been accomplished even on much smaller and more accessible islets elsewhere—perhaps the need requires to be demonstrated more clearly than has yet been the case in Dr Bellamy’s television film about his previous expeditions.

From the information available so far (Bourne, W.R.P., *Atoll Res. Bull.*, 149, 175–207; 1971), there appears to be nothing particularly unusual about the islands in question. While there has been a previous report that Abbott’s Booby *Sula abbotti* has been seen at sea in the area, there is an immature plumage of the Masked Booby *Sula dactylatra* which strongly resembles that of adult Abbott’s Boobies, and it would be helpful if more details of the birds seen could be provided to confirm their identity. If Abbott’s Booby

were found nesting in the group, this could be of crucial importance for the species, which is now reduced to about 2,000 pairs threatened by phosphate mining on Christmas Island (Nelson, J.B., *J. mar. biol. Ass. India*, 14, 643–662; 1974). On the other hand, although rats have reached Christmas Island they have not harmed the tree-nesting boobies there yet, although the birds might be more vulnerable in the lower vegetation of the Chagos group. The case for regarding the western Chagos islands as a potential refuge for this remarkable booby has still to be proved.

The other seabirds frequenting the Chagos group are all widespread species. Despite statements in the film that the Masked Boobies show unusual features, the specimen from the Chagos group deposited in the British Museum (Natural History) appears to us to fall within the normal range of variation for the western Indian Ocean. Together with the smaller seabirds its numbers seem more likely to be limited by conditions at sea than on land, and it seems likely the birds can still find ample breeding-sites on the undisturbed, rat-free outer islets, while the few landbirds now present in the archipelago appear to be introduced. It therefore seems questionable whether the rats are causing any more harm to the birds than to the vegetation.

While we yield to nobody in our concern at the harm caused by introduced mammals on islands (Bourne, W.R.P., *New Scientist*, 67, 422–425; 1975), we feel that it might be more useful if before they try to exterminate the rats Dr Bellamy and his colleagues were to dispose of the donkeys left on Diego Garcia, Ile du Coin and Ile Boddam elsewhere in the Chagos group at the time of their enforced evacuation in the early 1970s (Curtis, W.F., *Sea Swallow*, 25, 11–13; 1976). These seem likely to present a really serious threat to the regenerating natural vegetation if they are left to increase. It would also be interesting to know what arrangements our authorities have made to maintain continuing scientific surveillance of these potentially important nature reserves now that they have evicted their human inhabitants.

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J. B. NELSON

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news and views

Transient galactic radio and X-ray sources

from F. G. Smith

DETAILED correlation between the radio and the X-ray emission of celestial sources is hardly to be expected in view of the different radiation mechanisms involved at such widely different parts of the spectrum. A general correlation might, however, be expected from the association both of X-ray and radio emission with unusually energetic objects, some of which are variable. Some such are now turning up.

Many galactic X-ray sources are variable, but rather few are also radio sources. A large part of a single issue of *Nature* was recently devoted to the source Cyg X-3, which has given a series of very large radio outbursts which were not related in detail to its X-ray variations. Another example, A0620-00, gave an X-ray outburst which again was detected at a wide range of radio frequencies (Little *et al.*, *Nature*, **261**, 113; 1976); here the radio variations followed fairly closely the X-ray flux. The problem in interpreting the radio emissions is to extend observa-

tions to low enough frequencies to establish the spectrum, and in particular to look for the self-absorption typical of synchrotron radiation. Observationally, this means detecting a very weak source among the multitude of others, and finding a sufficiently accurate position for the identification with the X-ray source despite the problems of position-finding at long radio wavelengths.

The transient radio source reported by Davies *et al.* (page 476 of this issue of *Nature*) was found during a study of the galactic centre, using the high resolution interferometers at Jodrell Bank, operating at 960 MHz and 408 MHz. The source was detectable for one day only, but fortunately it was sufficiently near a known radio source for a position to be found to within 3 arc s. The position fits well with that of the X-ray source A1742-28, and incidentally improves the positional accuracy of that source by over 500 times (expressed as an area).

The meagre spectral information

given by this one result suggests that this source is more like A0620-00 than Cyg X-3. It would be useful to find it optically, but there is not much hope of this in the crowded and obscured galactic centre region. The accurate position will, however, make the search worth trying.

Many X-ray sources are known to be members of close binary systems, in which the atmosphere of a compact star is heated by the transfer of material from a giant. The tantalising glimpses which we now have of the radio and X-ray variables suggest that another type is involved, in which the energy source is more sporadic. In the centres of globular clusters, for example, there might be collisions or near-collisions between stars, giving rise both to heated dense atmospheres for the X rays and much more extended atmospheres, probably containing magnetic fields as well as charged particles, which give the radio emission. The proximity of this source to the galactic centre will encourage thought on such lines. □

Space probes *versus* comets

from David W. Hughes

THE fringes of the Solar System obviously contain mass, any comet with a period greater than 300 years spending a considerable fraction of its life beyond the orbit of Pluto. Öort (*Bull. Astron. Inst. Neth.*, **11**, 91; 1950) went so far as to propose the existence of an enormous cloud of comets with aphelias some tens of thousands of a.u. from the Sun. Detecting this mass is the problem. In 1972 Axford proposed that the cometary belt could be detected by observing its gravitational effect on a spacecraft which is headed out of the Solar System. A. P. Boss and S. J. Peale (Department of Physics, University of California) show, however,

that the secular perturbations of the orbits of long-period comets are currently three orders of magnitude more sensitive than spacecraft for detecting an unseen distribution of cometary or other masses near the outer regions of the Solar System (*Icarus*, **27**, 119; 1976).

The authors produce specific models for the mass distribution and then, using a numerical integral computation, find the acceleration that this mass distribution gives to a space probe as it moves through the Solar System. The velocity of the spacecraft along the line of sight can be easily obtained from the Doppler shift in the frequency of the trans-

mitted radio signal. A one minute integration of the Doppler data enables the line of sight velocity to be measured to an accuracy of ± 0.03 cm s⁻¹. Temporal variations in this velocity give the acceleration. Most spacecraft, including the deep space probes Pioneer and Mariner, suffer from outgassing and other variable effects which produce stray accelerations. These can be as high as 10^{-7} cm s⁻² and random in direction. This uncertainty leads to a total minimum detectable cometary-cloud mass of the order of a thousand Earth masses.

Now Halley's comet has a period of about 76 years and an aphelia at about 35 a.u. Hamid, Marsden and

Whipple (*Astr. J.*, **73**, 729; 1968) analysed the orbits calculated from the 1835 November return and the 1909 October return and found that the argument of perihelion had increased by 1.039° , the ascending node by 1.045° and the inclination had decreased by 0.041° during this period. These orbital changes could have been caused by the gravitational effect of a hypothetical ring of matter in the ecliptic plane of the Solar System, this ring having a mass of 0.5 Earth masses and a radius of 40 a.u., or alternatively a mass of 1.3 Earth masses at a radius of 50 a.u., or a larger mass still at a larger radius. It now seems certain, however, that the long term motion of Halley is also significantly affected by "non-gravitational" forces. These are, in the main, caused by the rotation of the cometary nucleus coupled with the monodirectional incident radiation flux leading to an asymmetric efflux of gas and a resultant force in the plane of the orbit.

The problem is that the exact magnitude of the non-gravitational force effect is unknown, the results given above for the possible mass of a comet belt thus becoming upper limits.

Comets still have, though, an enormous (approximately one thousand times) sensitivity advantage over space probes when it comes to detecting hidden distributed mass in the Solar System, even though their effectiveness is decreased by unknown non-gravitational forces. The only way a space probe could overcome this advantage is by being especially designed to be clean and gas free and by being solely dedicated to the specific task of mass detection. □

Phytochrome and gibberellins

from John L. Stoddart

WHEN Jack watched the beanstalk climbing into the heavens he was probably witnessing one of the earliest recorded instances of gibberellin-stimulated extension growth and many plant physiologists have since wished that he could have found some way to bequeath his unique mutant to posterity.

Gibberellins are amongst the most potent of the naturally occurring plant growth regulators and exert maximal activity in most tissues when present in sub-microgram quantities. In the 38 years since the first crystalline isola-

tion of a gibberellin by Yabuta and Sumiki (*J. agric. Chem. Soc. Japan*, **14**, 1526; 1938) there has been a steady, and more recently rapid, increase in our knowledge of the structural variations which can occur (almost 50 gibberellins have been characterised to date) and the ways in which plants synthesise or degrade these compounds. On the other hand, although gibberellins are now used extensively as malting accelerators in the brewing industry and as fruit-setting agents for seedless grapes, we are still largely ignorant of the precise means whereby these highly active compounds influence plant growth.

In many cases there are clear relationships between light, expressed either as photoperiod or spectral composition, and responsiveness to gibberellins with some of the most intriguing observations being related to red/far-red (phytochrome dependent) light treatments. The nature of the relationship between gibberellins and the photomorphogenic pigment, phytochrome, in cereals has been examined in a recent series of publications on gibberellins in the plastids of dark-grown leaves (etioplasts).

When dark-grown cereal leaves are exposed to red light (660 nm) for as little as 15 min, very large increases occur in the extractable gibberellin content (Reid *et al.*, *Nature*, **217**, 580; 1968) and this response is known to require active RNA and protein synthesis. Up to 60% of the total gibberellin content of the leaf can be accounted for in terms of the biological activity contained in the chloroplast fraction (Stoddart, *Planta (Berl.)*, **81**, 106; 1968) and thus it is reasonable to look to the plastid for light-mediated effects on gibberellin activity. Using isolated etioplast preparations, Cooke and Saunders (*Planta (Berl.)*, **123**, 299; 1975) have shown that the red light effect on extractable biological activity could be solely attributed to the plastid and that no comparable increase occurred in other cell fractions, although these contained measurable amounts of gibberellin in both light and dark. Phytochrome was detected in the etioplast preparations (Cooke *et al.*, *Planta (Berl.)*, **124**, 319; 1975) and the increased biological activity was attributable to changes in at least two gibberellin-like components. The latter term is used when describing activity measurements based upon bioassays and where the active compound has not been chemically characterised.

These findings have been confirmed recently by Evans and Smith (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 138; 1976) using carefully authenticated etioplasts prepared by similar procedures. Their results strengthened the causal nature of the co-occurrence of gibberellins

and phytochrome by demonstrating that the enhancement of biological activity was far-red (730 nm) reversible.

Both groups have suggested that phytochrome could act by regulating the permeability of the plastid envelope to gibberellins, with the inference that this allows access to cytoplasmic or wall-bound action sites: an interpretation supported by the recent isolation of enriched envelope preparations containing small quantities of phytochrome (Evans and Smith, *Nature*, **259**, 323; 1976). The pigment content of this fraction was low when compared with that of the original homogenate (<1%) but no other etioplast subfractions contained measurable amounts. It is possible, however, that the pattern of associations could change after red irradiation. How then might envelope permeability changes account for the observed increase in plastid gibberellin activity after 660 nm irradiation? There are two possibilities. Evans and Smith propose that the first response is an efflux of gibberellin resulting in internal depletion which, in turn, stimulates biosynthesis by way of a feedback activation mechanism. There is evidence that chloroplasts do indeed have a gibberellin biosynthetic capacity (Raiton and Reid, *Plant Sci. Lett.*, **3**, 303; 1974) and Cooke *et al.*, showed that gibberellin biosynthesis inhibitors greatly reduced the red light response. The available inhibitory compounds are, however, of suspect specificity and some are known to have more general effects on protein synthesis.

An alternative explanation, initially advanced by Cooke and Saunders, is that increased biological activity reflects release from a 'bound' form (attachment to either proteins or membranes) within the plastid and, in this case, effects upon envelope permeability would be a secondary correlated process facilitating efflux of the released gibberellin. Data presented by Evans and Smith showing that ultrasonic treatment of unirradiated etioplast preparations can give a three-fold increase in extractable biological activity are possible support for this mechanism. In addition to rupturing the envelope, such treatments would be expected to solubilise material associated with lipoprotein or membranes. The rapidity of the red light effect is also consistent with the binding hypothesis.

Localisation of phytochrome in the envelope indicates that this may be a primary site of action but it is still uncertain whether the increased extractable gibberellin-like activity is produced by *de novo* synthesis using stored precursors or is the result of release from a bound pool. Further studies on gibberellin localisation and synthetic capacity in etioplasts are

needed before this aspect can be resolved. It seems that we may now be in sight of a possible resolution of the apparent discrepancy inherent in the observation that applied gibberellin will give a massive growth stimulation in tissues containing quantities of extractable activity sufficient to sustain the maximum potential growth rate. There could also be an acceptable explanation for the interchangeability of red light and exogenous gibberellin in the control of leaf unrolling in dark-grown cereals (Loveys and Wareing, *Planta Berol.*, **98**, 117; 1971).

Compartmentalisation of endogenous plant growth regulators coupled with environmentally-mediated release mechanisms offers an attractive solution to these, and many other, problems of plant development. The prospects are exciting and there are indications that the existence of such a system for leaf gibberellins may soon be unequivocally established. □

Polyamines and growth control

from Robert Shields

ALTHOUGH it has been known for some time that polyamine levels alter with changing rates of cell proliferation, the precise role (if any) that these molecules have in cell regulation is still a mystery.

The first step in the synthesis of polyamines is the decarboxylation of ornithine to form putrescine by ornithine decarboxylase (ODC) which is the rate limiting enzyme for polyamine synthesis. This enzyme is somewhat unusual in that its activity in a given tissue can vary by more than 20-fold in different conditions and it must also hold the world record for the fastest vanishing mammalian enzyme activity with a half life that can be as short as five minutes in some conditions. It is then hardly surprising that ODC has attracted so much attention. The second enzyme of the pathway, S-adenosyl methionine decarboxylase (SAM decarboxylase) takes part in the conversion of putrescine to spermidine and the sequence is completed with the conversion of spermidine to spermine. Increased levels of ODC and SAM decarboxylase invariably accompany increased cell proliferation and indeed the levels of these enzymes may be very closely correlated with the specific growth rate of certain cells (Heby *et al.*, *J. Cell Physiol.*, **86**, 511; 1975). Presumably it is not the levels of these enzymes themselves that may regulate growth but rather the polyamines they

produce. Careful analysis of polyamine concentrations and growth rate show that levels of spermidine seem to be closely linked with cell proliferation rates whereas putrescine and spermine show little or no correlation (Heby *et al.*, *op. cit.*). No amount of correlative evidence can prove a regulatory role for polyamines however; clearly a more direct approach is necessary.

If polyamines are growth regulatory then interfering with their synthesis by use of enzyme inhibitors should interfere with cell growth. And this is what happens; cell division proceeds more slowly and in some conditions may cease altogether. Two types of inhibitors have been used; one is the anti-leukaemic agent MGBG which inhibits SAM decarboxylase and the others are various ornithine analogues which inhibit ODC. Using MGBG Fillingame and Morris were able to block the increases in spermidine and spermine that are normally observed after stimulation of lymphocytes by concanavalin A (con A). The increased synthesis and processing of RNA and synthesis of protein brought about by con A continued unimpeded however (*Biochemistry*, **12**, 4479; 1973).

This important observation shows that increases in spermidine and spermine are not necessary for increases in protein and RNA synthesis although a requirement for certain basal levels of polyamines in macromolecular synthesis cannot be ruled out. Although MGBG had no effect on the increased synthesis of RNA and protein the drug could partially inhibit DNA synthesis. The inhibition was somewhat unusual in that the number of lymphocytes that entered DNA synthesis after con A stimulation was unaltered but progress through S phase to mitosis was impaired (Fillingame *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4042; 1975). These results suggested that increased levels of spermine or spermidine might be necessary for normal progress from DNA synthesis to cell division. One possible objection to these experiments is that blocking SAM decarboxylase with MGBG leads to elevation of putrescine levels which could fulfil the functions of spermidine and spermine sufficiently well for increased RNA and protein synthesis but not for DNA synthesis to occur. If this were the case it might be expected that blocking putrescine formation and hence the synthesis of spermidine and spermine might have more profound effects than those obtained with MGBG.

Precisely this line of approach is taken in recently reported experiments using α -methyl ornithine to inhibit ODC and hence polyamine synthesis (Mamont *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **173**, 1626; 1976). These workers used a suspension culture of a hepa-

toma cell line (HTC) whose growth rate is slowed at high cell densities; diluting the culture leads to a rise in ODC and polyamine levels followed by increased rates of DNA synthesis and cell division. If the culture is diluted in the presence of α -methyl ornithine, the cells enter DNA synthesis and divide normally but subsequent rounds of DNA synthesis and cell division are inhibited and cell proliferation ceases. Examination of polyamine levels after culture dilution shows that putrescine concentrations follow the activity of ODC (McCann *et al.*, *Biochem. biophys. Res. Commun.*, **64**, 336; 1975), peaking some time before the increase in DNA synthesis. In the presence of α -methyl ornithine putrescine concentrations fall very rapidly but no inhibition of DNA synthesis occurs until the spermidine levels have dropped significantly by which time the cells would normally have entered the second wave of cell division. The failure of the cells to enter this second round of cell division can be directly related to low spermidine levels as spermine concentrations remain relatively unchanged. Taken together with the results from lymphocytes and the close correlation between spermidine levels and growth rate it seems that of the polyamines only spermidine may play a part in DNA synthesis and cell division. A certain basal level of polyamines may be necessary for normal growth, however, and experiments using inhibitors of polyamine synthesis and the selection of cell mutants with altered polyamine metabolism may shed some light on the normal functions of these curious compounds. □



A hundred years ago

THE comparatively infrequent employment of electric light, considering the great success achieved in its production, would at first sight appear to be due to something in the application of the electricity itself. It has been repeatedly and satisfactorily proved that a continuous and powerful light can be produced by electricity, and the question naturally arises, Why is it not more frequently employed for practical purposes?

Unquestionably the first experiments with electric light were not successful, but this is generally the case with new inventions. Unfortunately, however, a feeling seems to have arisen directly against the application of electricity for lighting purposes, or at any rate against the employment of the existing apparatus in the hope that more perfect may soon be invented.

from *Nature*, **14**, 133-144, June 8, 1876

Reactions to nuclear anti-analogue states

from P. E. Hodgson

REACTIONS to isobaric analogue states have been extensively studied ever since their discovery by Anderson and Wong in 1962, and have yielded much useful information on nuclear structure and on the nucleon optical potential (*Nature*, **247**, 179; 1974). More recently, it has proved possible to detect reactions to anti-analogue states, and analyses of their cross sections are giving interesting results.

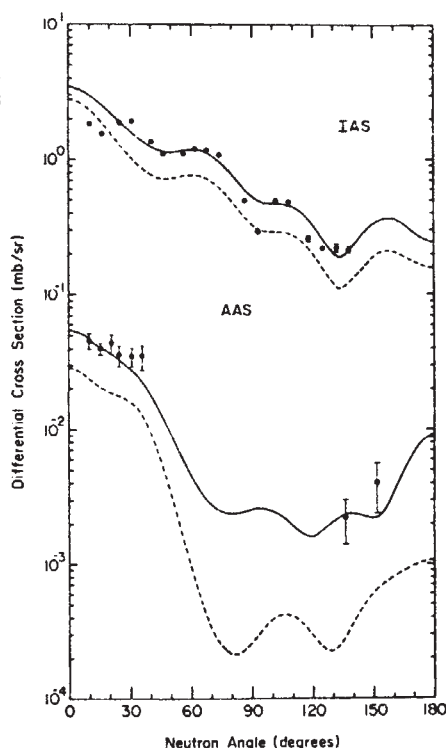
The simplest reaction to these states is the (p,n) reaction, that adds a proton and removes a neutron from the ground state of the target nucleus. The final nucleus thus has the same number of nucleons as the target nucleus and essentially the same mass; this is why the final states are called 'isobaric'. At the same time the charge has been increased by one, and the ways of doing this can most easily be described using the isobaric spin formalism, which is exactly similar to the corresponding formalism for ordinary spin. Each nuclear state has an isobaric spin, with three components like a spin vector. The third component T_3 is directly related to the nuclear charge, $T_3 = \frac{1}{2}(N-Z)$, where N and Z are the numbers of neutrons and protons in the nucleus. All the states of a nucleus thus have the same value of T_3 , but they can have any total isobaric spin equal to or greater than T_3 , since no vector can be shorter than any one of its components.

The ground states of nuclei nearly always have $T=T_3$, so when we increase the charge by one by a (p,n) reaction we reduce T_3 by one, since by convention a neutron has $T_3 = \frac{1}{2}$ and a proton has $T_3 = -\frac{1}{2}$. The final states can thus have a total isobaric spin of T or $T-1$. The state with isobaric spin T is the isobaric analogue state, and apart from small electromagnetic effects it has exactly the same structure as the target ground state, except of course for the reduced charge. All the nucleons are coupled together in the same way, and we can say that the only difference is that one isobaric spin vector has been flipped so that its third component is $-\frac{1}{2}$ instead of $\frac{1}{2}$. Quantum mechanically the states have a very high overlap, and so reactions from one to the other take place easily, and it is their high cross sections that enable them to be detected even though many other reactions were also taking place.

The anti-analogue state with isobaric spin $T-1$ is just the opposite; quantum mechanically its structure is nearly orthogonal to that of the ground state

of the target. Reactions to this state are thus very difficult, and can only take place at all because of small differences in structure from complete orthogonality. Unlike the analogue state reactions, the anti-analogue state reactions are sensitive to small components of the wavefunctions, and they are thus difficult to describe theoretically as well as being difficult to detect due to their low cross sections.

Early work on anti-analogue state reactions used the ($^3\text{He}, t$) reaction, which also has the net effect of adding a proton and removing a neutron. Calculations of the cross section of the reaction on ^{40}Ar by Schaeffer and Bertsch (*Phys. Lett.*, **38B**, 159; 1972) showed that the direct component can only account for a very small fraction of the observed cross section, but that a reasonable fit can be obtained by assuming that the reaction takes place mainly by a two-step process in which a ($^3\text{He}, \alpha$) reaction is rapidly followed by a (α, t) reaction. Similar results were obtained for the ($^3\text{He}, t$) reaction on ^{48}Ca .



Differential cross sections of the $^{40}\text{Ar}(p,n)^{40}\text{K}$ reaction at 24 MeV to the isobaric analogue and anti-analogue states compared with distorted wave calculations assuming that the reactions take place in one step. The dashed curves show the result of the direct transition only and the solid curves the result of including the knock-on exchange contributions. The isobaric analogue state cross sections were obtained by Bentley and colleagues (*Phys. Rev. Lett.*, **27**, 1081; 1971).

Studies have now been made of the (p,n) reaction to the anti-analogue state of ^{40}Ar by Galonsky and colleagues of Michigan State University (*Phys. Rev. Lett.*, **35**, 1208; 1975). They used 24 MeV protons, and were able to detect the very small cross sections of the anti-analogue state reaction using a large liquid scintillator and a time-of-flight spectrometer. As shown in the figure, these cross sections are only about 1% of the corresponding analogue state ones.

They analysed these data using the distorted wave theory assuming that the reaction takes place on one step, and including both the direct and the knock-on exchange contributions. The dashed curves in the figure are for the direct process only and the solid curves show the result of including exchange. The parameters of the distorting potentials and of the effective nucleon-nucleon interaction were all taken from previous work.

It is clear from the figure that this calculation gives cross sections in very good agreement with the experimental data, showing that at least in this case it is not necessary to take into account the possibility of two-step reactions in which the incident and outgoing particles are composite, for then the exchange processes are expected to be more likely than the direct.

The relative probabilities of one-step and multi-step processes is a very interesting question, and it is likely that further studies of reactions to anti-analogue state will provide important information. They should also tell us more about the smaller components of the nuclear wavefunctions. □

Mammalian DNA repair

from a Correspondent

The Second International Workshop on DNA Repair Mechanisms in Mammalian Cells was held at Nordwijkerhout, the Netherlands, on May 2-5.

ALL cellular systems that have been examined possess repair mechanisms for dealing with lesions introduced into DNA. The general purpose of investigations on mammalian DNA repair and of the Second International Workshop on DNA Repair Mechanisms in Mammalian Cells has been to understand how damage in cellular DNA is recognised and removed—or alternatively bypassed—and the effect of the processes involved (that is, mutation or cancer).

The inability to carry out normal repair or DNA metabolism is associated with several human diseases, which were summarised by J. German III (New York Blood Center). Among the diseases studied extensively are xeroderma pigmentosum (XP) and ataxia telangiectasia (AT), both extremely rare. Cells from affected individuals lack the first step in removing lesions induced in their DNA by ultraviolet light or ionising radiation, respectively, which correlates well with the sensitivity of these individuals to sunlight or ionising radiation. There are now five complementation groups for XP and at least two for AT, based largely on the cell fusion studies of D. Bootsma (Erasmus University, Rotterdam), P. Lohman (Medical Biological Laboratories, Rijswijk) and M. Paterson (Atomic Energy of Canada, Chalk River). Although XP cells lack the ability to excise ultraviolet-light-induced pyrimidine dimers from their DNA *in vivo*, the excising function is still present. As shown by E. Friedberg (Stanford University Medical Center), the dimers are removed from "naked" DNA added to crude extracts of these cells. Since extracts of cells from normal individuals can carry out DNA repair on chromatin from irradiated XP cells and XP extracts cannot, it was concluded that repair must be considered at the level of chromosome structure as well.

A DNA unwinding protein may be important for repair at the chromosome level. A. Huang (Duke University Medical Center) has isolated such a protein from lymphocytes of patients with chronic lymphocytic leukaemia. It enhances the first step in pyrimidine dimer removal, by repair enzymes, causes a decondensation of chromatin, and is present at about three to five times the concentration observed for normal cells. Furthermore, there is considerably more repair synthesis after ultraviolet irradiation of cells from patients with this leukaemia compared with normal cells.

Another type of repair has been identified, first in bacteria and then in mammalian cells, which allows semi-conservative replication past pyrimidine dimers in DNA. Frequently gaps are left in the DNA and these are filled in later either by *de novo* synthesis or, based on the work of R. Meneghini (University of San Paulo) and A. Lehmann (MRC Mutation Unit, University of Sussex), by possible exchange processes which can result in dimers appearing in DNA synthesised after irradiation. Approximately 20% of the dimers appear in new DNA. The reassortment may correlate with the high frequency of sister chromatid exchanges which result after ultraviolet irradiation of cells from XP patients as

observed by de Weerd-Kastelein (Erasmus University, Rotterdam). Molecular evidence for recombination of mammalian DNA was presented by P. Moore (National Institute for Medical Research, London). He reported that a small proportion of DNA recovered from cells treated with mitomycin C (a carcinogen that in fungi is also a potent recombinogen) has the hybrid intermediate characteristic of DNA undergoing recombination. The importance of being able to carry out genetic recombination was emphasised by the work with lower eukaryotes summarised by M. Resnick (National Institute of Medical Research, London). Evidence was presented that haploid organisms, with only one copy of their genome, are much more sensitive to ionising radiation than those which are diploid (including mammalian cells) or haploid but with a duplicated genome; he suggested that this may be due to the capacity to undergo recombination.

Some of the gaps which occur in newly synthesised DNA after exposure to ultraviolet light have been suggested by S. Sedgwick (Laboratoire d'Enzymologie, Gif-sur-Yvette) to be the sites of very high levels of mutation. An inducible protein which may be error prone has been proposed by M. Radman (University of Brussels) to be part of an "SOS" repair system in bacteria. Evidence from his laboratory indicates that a protein is induced in a mutant strain of *E. coli* that promotes misincorporation of nucleotides into DNA *in vitro*.

Prompted by the work in bacteria, investigations have begun on the appearance of inducible proteins after mutagen treatment of animals or mammalian cells. R. Hart (Ohio State University Hospital, Columbus) has identified a protein fraction from whole animals and from cells in culture that appears after treatment with ultraviolet light or target carcinogens (those which act on specific tissues; for example, 3-methyl chloranthracene acts specifically on lung tissue). This protein is similar to the "protein x" from bacteria in that it has a molecular weight of 40,000 and is inducible by mutagen treatment.

The lesions induced by irradiation and mutagen treatment were the subject of considerable discussion. Many studies have focused on assaying the repair of single types of damage such as pyrimidine dimers produced by ultraviolet light or breaks in DNA induced by ionising radiation. P. Cerutti (University of Florida, Gainesville) summarised observations from various laboratories which have shown that several types of lesions may be produced by a given agent. It was apparent that considerable caution should be used when drawing conclusions about the repair of cellular DNA based on the

changes in only a single category of lesions. □

High resolution NMR of glasses

from Paul Calvert

CURRENT attempts to produce high resolution spectra of solid polymers are among the most sophisticated and, to the outsider, incomprehensible experiments in polymer science. Results from this technique are now being used to explain results from some of the crudest measurements, namely impact strength measurements. The development of high resolution NMR for isotropic solids means that the motion of any carbon atom or group in a polymer can be measured as a function of frequency and temperature. This is a considerable advance over wide line NMR which cannot distinguish which groups are moving and should tell us a great deal about the structure and deformation of polymers.

High resolution NMR spectroscopy of solids has seemed imminent since Pulse Fourier Transform (PFT) spectrometers became widely available around 1971. Using PFT, spectra from ^{13}C nuclei in natural abundance could be obtained which were well resolved even for quite viscous samples such as liquid polymers, gels and rubbers (for example see Schaeffer, *Macromolecules*, **4**, 110; 1971; and **5**, 427; 1972). In solids, interactions between neighbouring carbon and hydrogen nuclear magnetic dipoles are not averaged out by molecular motion and broaden the spectral lines into oblivion.

One way to avoid this is to use a very strong hydrogen decoupling field to magnetically stir up the protons thoroughly so that there is no net interaction with the carbons. Things may be improved further by using cross polarisation whereby rather than irradiating the carbons directly they are driven by setting the protons spinning at the appropriate frequency and using the internal field from them to drive the carbons (Pines, Gibby and Waugh, *J. chem. Phys.*, **56**, 1776; 1972; and **59**, 569; 1973). For most polymers the end results are disappointing; lines can be seen but are broad enough to overlap. This is mainly due to chemical shift anisotropy, the fact that interactions within the molecule make the line position depend on the orientation of the molecule to the magnetic field and with no molecular motion there is no averaging.

An alternative method to narrow the lines is to spin the sample at the "magic angle" 54.7° to the field. At this angle $3\cos^2\theta - 1$ is zero and dipolar couplings and chemical shift anisotropies are averaged out. Schneider and coworkers in Prague report the application of this method to polyethylene (*Macromolecules*, **5**, 120, 125; 1970). A spin rate of up to 12 kHz can be produced using an air jet to drive a sample in the shape of a turbine, but for complete narrowing 50 kHz would be needed.

J. Schaeffer of Monsanto, St Louis has described a combination of these two techniques, using strong decoupling and cross polarisation to remove the high frequency part of the interactions and spinning at 3 kHz to remove what was left. The first published results (*Macromolecules*, **8**, 291; 1975) were not overwhelming but those presented at the American Physical Society meeting in Atlanta last month are very impressive (see *Bull. Am. Phys. Soc. Series 11*, **21**, 443; 1976). Spectra with a resolution of 2 p.p.m., comparable with those of solutions, were shown for a range of glassy synthetic polymers, ebony and ivory though the last was not resolved as the spectrum is too complex.

Measurement of several relaxation rates associated with the NMR experiment provides information about molecular motions involving each of the carbon atoms in the MHz, kHz and Hz regions. Before, this was only available for the structure as a whole (for a recent review of broad line NMR see McBrierty, *Polymer*, **15**, 503; 1974). This will have a marked effect on our understanding of the glass transition and those secondary transitions in glasses which involve cooperative molecular motions.

In contrast to the sophisticated and expensive NMR apparatus, impact strength (toughness) is usually measured as the energy absorbed when a swinging hammer breaks a sample (a hundred dollars would buy a good one). The fracture process could be characterised by a time of about 100 μ s, that is molecular motions at a frequency of 10 kHz. There have been many attempts to correlate this important property with structure and molecular motion but none is very convincing.

Vincent (*Polymer*, **15**, 111; 1974) has demonstrated a correlation of impact strength with dynamic modulus and mechanical loss but only by dividing the impact behaviour into four categories rather than using numerical data. One case that particularly demands explanation is polycarbonate, an exceptionally tough glassy polymer which loses its toughness on annealing. Schaeffer showed at the Atlanta meeting that for seven glassy polymers impact strength increased monotonically

with the ratio of NMR relaxation time for main chain carbons at low frequency (T_{CH}) to that at around 10 kHz ($T_{1\rho}$). In other words, if a molecule can relax easily at 10 kHz it has a high impact strength. Polycarbonate had the highest value of $T_{CH}/T_{1\rho}$ and this decreases by $2\frac{1}{2}$ times on annealing. Discussion of the connection between these motions and impact strength is still at the hand waving stage, but it is suggested that the plastic work of fracture which accounts for the energy absorbed on impact depends on slip between chains which in turn depends on the inter- and intra-chain interactions that govern the motions associated with $T_{1\rho}$. $\square$

Predicting volcanic eruptions

from Peter J. Smith

A DISTINGUISHED British volcanologist recently claimed in a radio broadcast that most volcanic eruptions could be predicted if only adequate funds were to be made available for monitoring equipment. But although the prediction of volcanic activity may be easier than the prediction of earthquakes, knowledge of eruptive precursors is still far from complete. Moreover, as Weaver and Malone (*Geophys. Res. Lett.*, **3**, 197; 1976) now point out, even in existing types of data there may be more than meets the eye, especially where glacier-clad volcanoes are concerned.

The phenomenon that Weaver and Malone have in mind is seismic events of low frequency (predominant frequency content less than 5 Hz). Such events have been observed for a number of years in Japan where Minakami *et al.* (*Bull. Earthquake Res. Inst.*, **47**, 893; 1969) termed them type B events. They usually occur within 1 km of a volcanic crater and are apparently associated with eruptive and pre-eruptive processes, for in some cases they have been observed to increase in frequency both before and during actual eruptions. This has led to their being used to predict eruptions from some volcanoes, even though their precise origins are obscure.

Type B events have now been recorded in the vicinity of volcanoes in several parts of the world outside Japan, including Alaska, Indonesia and Central America. Apparently identical events have also been observed near quiescent volcanoes in the Cascade Range of Washington state (see, for

example, Unger and Decker, *Bull. Seismol. Soc. Amer.*, **60**, 2023; 1970). Because of the obvious potential of such events for eruption prediction, Weaver and Malone have carried out a more thorough study of those associated with Mt Saint Helens, an historically active stratovolcano in the Cascade Range.

Of the thousands of low frequency events recorded, only a few hundred have yet been analysed in any detail. But all were found to have occurred on the glacier-clad north-eastern side of the mountain and all had very shallow depths (<200 m). In these respects, as well as in waveform characteristics, they were therefore easily distinguishable from the deeper (1–3 km) and much less frequent tectonic earthquakes. From this and related evidence, Weaver and Malone conclude that there is a 'compelling' case for attributing the low frequency events not directly to volcanic activity but to glacial movement ('glacier noise'). Indeed, two events studied in particular detail were found to have occurred at a depth of 45 ± 20 m, which is roughly the thickness of the ice in the areas concerned.

If Weaver and Malone are right, there are clearly going to be problems in using type B events for eruption prediction, at least for Mt Saint Helens. But Mt Saint Helens is quiescent, and so the question of prediction is presumably not urgent. By contrast, Mauna Loa, the largest of the five volcanoes making up the island of Hawaii, is in a quite different position.

Mauna Loa has been created by repeated eruptions over several hundred thousand years and during the past century or so has erupted at irregular intervals averaging 3–4 yr. From 1950 to 1975, however, it never erupted at all, although this 25-yr period of dormancy, the longest in the volcano's recorded history, was broken in July 1975. The 1975 eruption was small by Mauna Loa's standards, producing only 3×10^7 m³ of lava. But according to US Geological Survey scientists it was highly significant, for historically there has been a cyclic pattern of activity comprising two small summit eruptions followed by a large flank eruption.

Assuming this pattern to be repeated, there is now a specific prediction for the second brief summit and major flank eruptions to take place before July 1978. Moreover, seismic data suggest that the flank eruption will occur along the north-east rift. In this case there will be some threat to Hilo, the island's principal city, which lies about 50 km away but in the direction of natural drainage channels which could guide the lava. However, there are contingency plans to redirect the lava if necessary. $\square$

review article

Simple mathematical models with very complicated dynamics

Robert M. May*

First-order difference equations arise in many contexts in the biological, economic and social sciences. Such equations, even though simple and deterministic, can exhibit a surprising array of dynamical behaviour, from stable points, to a bifurcating hierarchy of stable cycles, to apparently random fluctuations. There are consequently many fascinating problems, some concerned with delicate mathematical aspects of the fine structure of the trajectories, and some concerned with the practical implications and applications. This is an interpretive review of them.

THERE are many situations, in many disciplines, which can be described, at least to a crude first approximation, by a simple first-order difference equation. Studies of the dynamical properties of such models usually consist of finding constant equilibrium solutions, and then conducting a linearised analysis to determine their stability with respect to small disturbances: explicitly nonlinear dynamical features are usually not considered.

Recent studies have, however, shown that the very simplest nonlinear difference equations can possess an extraordinarily rich spectrum of dynamical behaviour, from stable points, through cascades of stable cycles, to a regime in which the behaviour (although fully deterministic) is in many respects "chaotic", or indistinguishable from the sample function of a random process.

This review article has several aims.

First, although the main features of these nonlinear phenomena have been discovered and independently rediscovered by several people, I know of no source where all the main results are collected together. I have therefore tried to give such a synoptic account. This is done in a brief and descriptive way, and includes some new material: the detailed mathematical proofs are to be found in the technical literature, to which signposts are given.

Second, I indicate some of the interesting mathematical questions which do not seem to be fully resolved. Some of these problems are of a practical kind, to do with providing a probabilistic description for trajectories which seem random, even though their underlying structure is deterministic. Other problems are of intrinsic mathematical interest, and treat such things as the pathology of the bifurcation structure, or the truly random behaviour, that can arise when the nonlinear function $F(X)$ of equation (1) is not analytical. One aim here is to stimulate research on these questions, particularly on the empirical questions which relate to processing data.

Third, consideration is given to some fields where these notions may find practical application. Such applications range from the abstractly metaphorical (where, for example, the transition from a stable point to "chaos" serves as a metaphor for the onset of turbulence in a fluid), to models for the dynamic behaviour of biological populations (where one can seek to use field or laboratory data to estimate the values of the parameters in the difference equation).

Fourth, there is a very brief review of the literature pertaining to the way this spectrum of behaviour—stable points, stable cycles, chaos—can arise in second or higher order difference equations (that is, two or more dimensions; two or more interacting species), where the onset of chaos usually requires less severe nonlinearities. Differential equations are also surveyed in this light; it seems that a three-dimensional system of first-order ordinary differential equations is required for the manifestation of chaotic behaviour.

The review ends with an evangelical plea for the introduction of these difference equations into elementary mathematics courses, so that students' intuition may be enriched by seeing the wild things that simple nonlinear equations can do.

First-order difference equations

One of the simplest systems an ecologist can study is a seasonally breeding population in which generations do not overlap¹⁻⁴. Many natural populations, particularly among temperate zone insects (including many economically important crop and orchard pests), are of this kind. In this situation, the observational data will usually consist of information about the maximum, or the average, or the total population in each generation. The theoretician seeks to understand how the magnitude of the population in generation $t+1$, X_{t+1} , is related to the magnitude of the population in the preceding generation t , X_t : such a relationship may be expressed in the general form

$$X_{t+1} = F(X_t) \quad (1)$$

The function $F(X)$ will usually be what a biologist calls "density dependent", and a mathematician calls nonlinear; equation (1) is then a first-order, nonlinear difference equation.

Although I shall henceforth adopt the habit of referring to the variable X as "the population", there are countless situations outside population biology where the basic equation (1), applies. There are other examples in biology, as, for example, in genetics^{5,6} (where the equation describes the change in gene frequency in time) or in epidemiology⁷ (with X the fraction of the population infected at time t). Examples in economics include models for the relationship between commodity quantity and price⁸, for the theory of business cycles⁹, and for the temporal sequences generated by various other economic quantities¹⁰. The general equation (1) also is germane to the social sciences¹¹, where it arises, for example, in theories of

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learning (where X may be the number of bits of information that can be remembered after an interval t), or in the propagation of rumours in variously structured societies (where X is the number of people to have heard the rumour after time t). The imaginative reader will be able to invent other contexts for equation (1).

In many of these contexts, and for biological populations in particular, there is a tendency for the variable X to increase from one generation to the next when it is small, and for it to decrease when it is large. That is, the nonlinear function $F(X)$ often has the following properties: $F(0)=0$; $F(X)$ increases monotonically as X increases through the range $0 < X < A$ (with $F(X)$ attaining its maximum value at $X=A$); and $F(X)$ decreases monotonically as X increases beyond $X=A$. Moreover, $F(X)$ will usually contain one or more parameters which "tune" the severity of this nonlinear behaviour; parameters which tune the steepness of the hump in the $F(X)$ curve. These parameters will typically have some biological or economic or sociological significance.

A specific example is afforded by the equation^{1,4,12-23}

$$N_{t+1} = N_t(a - bN_t) \quad (2)$$

This is sometimes called the "logistic" difference equation. In the limit $b=0$, it describes a population growing purely exponentially (for $a > 1$); for $b \neq 0$, the quadratic nonlinearity produces a growth curve with a hump, the steepness of which is tuned by the parameter a . By writing $X = bN/a$, the equation may be brought into canonical form^{1,4,12-23}

$$X_{t+1} = aX_t(1 - X_t) \quad (3)$$

In this form, which is illustrated in Fig. 1, it is arguably the simplest nonlinear difference equation. I shall use equation (3) for most of the numerical examples and illustrations in this article. Although attractive to mathematicians by virtue of its extreme simplicity, in practical applications equation (3) has the disadvantage that it requires X to remain on the interval $0 < X < 1$; if X ever exceeds unity, subsequent iterations diverge towards $-\infty$ (which means the population becomes extinct). Furthermore, $F(X)$ in equation (3) attains a maximum value of $a/4$ (at $X=1/2$); the equation therefore possesses non-trivial dynamical behaviour only if $a < 4$. On the other hand, all trajectories are attracted to $X=0$ if $a < 1$. Thus for non-trivial

dynamical behaviour we require $1 < a < 4$; failing this, the population becomes extinct.

Another example, with a more secure provenance in the biological literature^{1,23-27}, is the equation

$$X_{t+1} = X_t \exp[r(1 - X_t)] \quad (4)$$

This again describes a population with a propensity to simple exponential growth at low densities, and a tendency to decrease at high densities. The steepness of this nonlinear behaviour is tuned by the parameter r . The model is plausible for a single species population which is regulated by an epidemic disease at high density²⁸. The function $F(X)$ of equation (4) is slightly more complicated than that of equation (3), but has the compensating advantage that local stability implies global stability¹ for all $X > 0$.

The forms (3) and (4) by no means exhaust the list of single-humped functions $F(X)$ for equation (1) which can be culled from the ecological literature. A fairly full such catalogue is given, complete with references, by May and Oster¹. Other similar mathematical functions are given by Metropolis *et al.*¹⁶. Yet other forms for $F(X)$ are discussed under the heading of "mathematical curiosities" below.

Dynamic properties of equation (1)

Possible constant, equilibrium values (or "fixed points") of X in equation (1) may be found algebraically by putting $X_{t+1} = X_t = X^*$, and solving the resulting equation

$$X^* = F(X^*) \quad (5)$$

An equivalent graphical method is to find the points where the curve $F(X)$ that maps X_t into X_{t+1} intersects the 45° line, $X_{t+1} = X_t$, which corresponds to the ideal nirvana of zero population growth; see Fig. 1. For the single-hump curves discussed above, and exemplified by equations (3) and (4), there are two such points: the trivial solution $X=0$, and a non-trivial solution X^* (which for equation (3) is $X^* = 1 - [1/a]$).

The next question concerns the stability of the equilibrium point X^* . This can be seen^{24,25,19-21,1,4} to depend on the slope of the $F(X)$ curve at X^* . This slope, which is illustrated by the dashed lines in Fig. 1, can be designated

$$\lambda^{(1)}(X^*) = [dF/dX]_{X=X^*} \quad (6)$$

So long as this slope lies between 45° and -45° (that is, $\lambda^{(1)}$ between +1 and -1), making an acute angle with the 45° ZPG line, the equilibrium point X^* will be at least locally stable, attracting all trajectories in its neighbourhood. In equation (3), for example, this slope is $\lambda^{(1)} = 2 - a$: the equilibrium point is therefore stable, and attracts all trajectories originating in the interval $0 < X < 1$, if and only if $1 < a < 3$.

As the relevant parameters are tuned so that the curve $F(X)$ becomes more and more steeply humped, this stability-determining slope at X^* may eventually steepen beyond -45° (that is, $\lambda^{(1)} < -1$), whereupon the equilibrium point X^* is no longer stable.

What happens next? What happens, for example, for $a > 3$ in equation (3)?

To answer this question, it is helpful to look at the map which relates the populations at successive intervals 2 generations apart; that is, to look at the function which relates X_{t+2} to X_t . This second iterate of equation (1) can be written

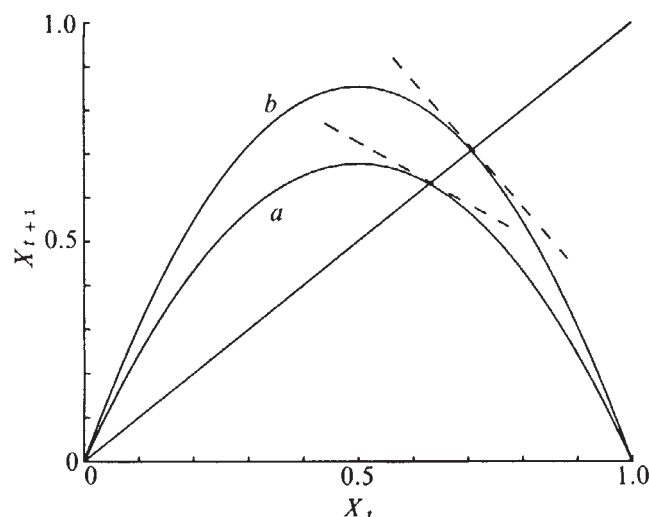
$$X_{t+2} = F[F(X_t)] \quad (7)$$

or, introducing an obvious piece of notation,

$$X_{t+2} = F^{(2)}(X_t) \quad (8)$$

The map so derived from equation (3) is illustrated in Figs 2 and 3. Population values which recur every second generation (that

Fig. 1 A typical form for the relationship between X_{t+1} and X_t described by equation (1). The curves are for equation (3), with $a = 2.707$ (a); and $a = 3.414$ (b). The dashed lines indicate the slope at the "fixed points" where $F(X)$ intersects the 45° line: for the case a this slope is less steep than -45° and the fixed point is stable; for b the slope is steeper than -45°, and the point is unstable.



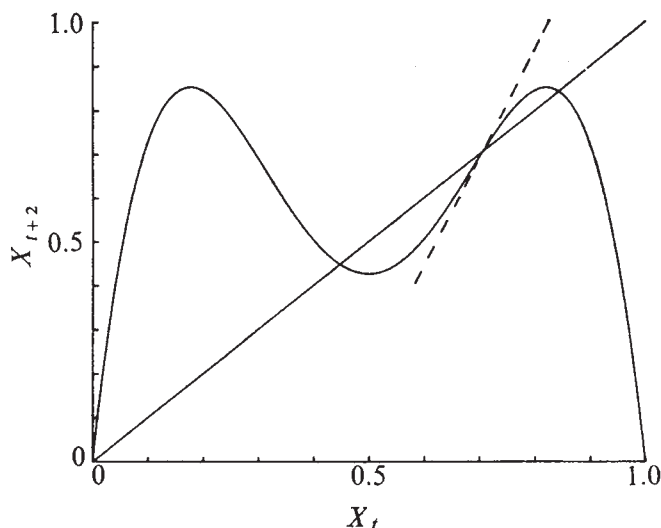


Fig. 2 The map relating X_{t+2} to X_t , obtained by two iterations of equation (3). This figure is for the case (a) of Fig. 1, $a = 2.707$: the basic fixed point is stable, and it is the only point at which $F^{(2)}(X)$ intersects the 45° line (where its slope, shown by the dashed line, is less steep than 45°).

is, fixed points with period 2) may now be written as X^*_2 , and found either algebraically from

$$X^*_2 = F^{(2)}(X^*_2) \quad (9)$$

or graphically from the intersection between the map $F^{(2)}(X)$ and the 45° line, as shown in Figs 2 and 3. Clearly the equilibrium point X^* of equation (5) is a solution of equation (9); the basic fixed point of period 1 is a degenerate case of a period 2 solution. We now make a simple, but crucial, observation¹: the slope of the curve $F^{(2)}(X)$ at the point X^* , defined as $\lambda^{(2)}(X^*)$ and illustrated by the dashed lines in Figs 2 and 3, is the square of the corresponding slope of $F(X)$

$$\lambda^{(2)}(X^*) = [\lambda^{(1)}(X^*)]^2 \quad (10)$$

This fact can now be used to make plain what happens when the fixed point X^* becomes unstable. If the slope of $F(X)$ is less than -45° (that is, $|\lambda^{(1)}| < 1$), as illustrated by curve *a* in Fig. 1, then X^* is stable. Also, from equation (10), this implies $0 < \lambda^{(2)} < 1$ corresponding to the slope of $F^{(2)}$ at X^* lying between 0° and 45° , as shown in Fig. 2. As long as the fixed point X^* is stable, it provides the only non-trivial solution to equation (9). On the other hand, when $\lambda^{(1)}$ steepens beyond -45° (that is, $|\lambda^{(1)}| > 1$), as illustrated by curve *b* in Fig. 1, X^* becomes unstable. At the same time, from equation (10) this implies $\lambda^{(2)} > 1$, corresponding to the slope of $F^{(2)}$ at X^* steepening beyond 45° , as shown in Fig. 3. As this happens, the curve $F^{(2)}(X)$ must develop a "loop", and two new fixed points of period 2 appear, as illustrated in Fig. 3.

In short, as the nonlinear function $F(X)$ in equation (1) becomes more steeply humped, the basic fixed point X^* may become unstable. At exactly the stage when this occurs, there are born two new and initially stable fixed points of period 2, between which the system alternates in a stable cycle of period 2. The sort of graphical analysis indicated by Figs 1, 2 and 3, along with the equation (10), is all that is needed to establish this generic result^{1,4}.

As before, the stability of this period 2 cycle depends on the slope of the curve $F^{(2)}(X)$ at the 2 points. (This slope is easily shown to be the same at both points^{1,20}, and more generally to be the same at all k points on a period k cycle.) Furthermore, as is clear by imagining the intermediate stages between Figs 2 and 3, this stability-determining slope has the value $\lambda = +1$ at the birth of the 2-point cycle, and then decreases through zero

towards $\lambda = -1$ as the hump in $F(X)$ continues to steepen. Beyond this point the period 2 points will in turn become unstable, and bifurcate to give an initially stable cycle of period 4. This in turn gives way to a cycle of period 8, and thence to a hierarchy of bifurcating stable cycles of periods 16, 32, 64, ..., 2^n . In each case, the way in which a stable cycle of period k becomes unstable, simultaneously bifurcating to produce a new and initially stable cycle of period $2k$, is basically similar to the process just adumbrated for $k=1$. A more full and rigorous account of the material covered so far is in ref. 1.

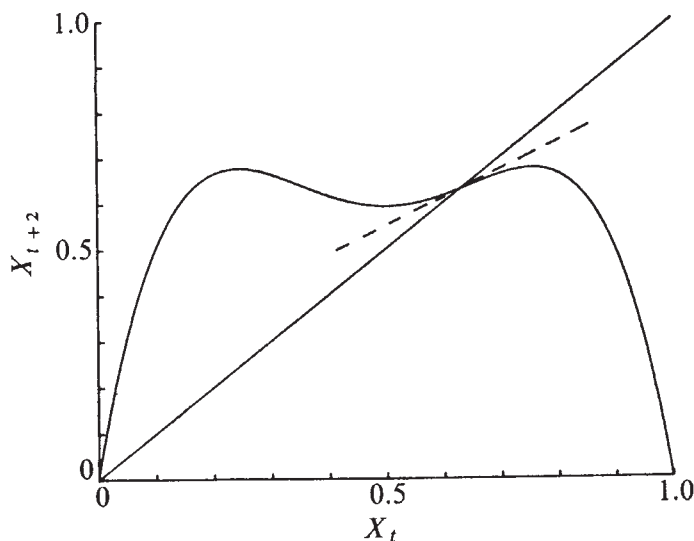
This "very beautiful bifurcation phenomenon"²² is depicted in Fig. 4, for the example equation (3). It cannot be too strongly emphasised that the process is generic to most functions $F(X)$ with a hump of tunable steepness. Metropolis *et al.*¹⁶ refer to this hierarchy of cycles of periods 2^n as the harmonics of the fixed point X^* .

Although this process produces an infinite sequence of cycles with periods 2^n ($n \rightarrow \infty$), the "window" of parameter values wherein any one cycle is stable progressively diminishes, so that the entire process is a convergent one, being bounded above by some critical parameter value. (This is true for most, but not all, functions $F(X)$: see equation (17) below.) This critical parameter value is a point of accumulation of period 2^n cycles. For equation (3) it is denoted a_c : $a_c = 3.5700$...

Beyond this point of accumulation (for example, for $a > a_c$ in equation (3)) there are an infinite number of fixed points with different periodicities, and an infinite number of different periodic cycles. There are also an uncountable number of initial points X_0 which give totally aperiodic (although bounded) trajectories; no matter how long the time series generated by $F(X)$ is run out, the pattern never repeats. These facts may be established by a variety of methods^{1,4,20,22,29}. Such a situation, where an infinite number of different orbits can occur, has been christened "chaotic" by Li and Yorke²⁰.

As the parameter increases beyond the critical value, at first all these cycles have even periods, with X_t alternating up and down between values above, and values below, the fixed point X^* . Although these cycles may in fact be very complicated (having a non-degenerate period of, say, 5,726 points before repeating), they will seem to the casual observer to be rather like a somewhat "noisy" cycle of period 2. As the parameter value continues to increase, there comes a stage (at $a = 3.6786$... for equation (3)) at which the first odd period cycle appears. At first these odd cycles have very long periods, but as the parameter value continues to increase cycles with smaller and smaller odd periods are picked up, until at last the three-point

Fig. 3 As for Fig. 2, except that here $a = 3.414$, as in Fig. 1b. The basic fixed point is now unstable: the slope of $F^{(2)}(X)$ at this point steepens beyond 45° , leading to the appearance of two new solutions of period 2.



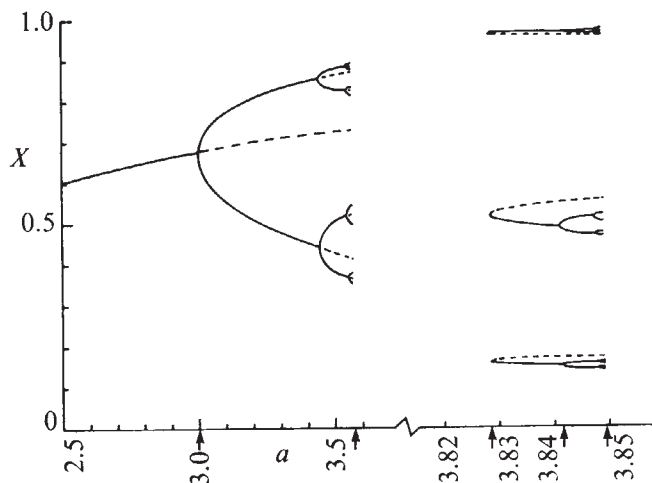


Fig. 4 This figure illustrates some of the stable (—) and unstable (---) fixed points of various periods that can arise by bifurcation processes in equation (1) in general, and equation (3) in particular. To the left, the basic stable fixed point becomes unstable and gives rise by a succession of pitchfork bifurcations to stable harmonics of period 2^n ; none of these cycles is stable beyond $a = 3.5700$. To the right, the two period 3 cycles appear by tangent bifurcation: one is initially unstable; the other is initially stable, but becomes unstable and gives way to stable harmonics of period 3×2^n , which have a point of accumulation at $a = 3.8495$. Note the change in scale on the a axis, needed to put both examples on the same figure. There are infinitely many other such windows, based on cycles of higher periods.

cycle appears (at $a = 3.8284 \dots$ for equation (3)). Beyond this point, there are cycles with every integer period, as well as an uncountable number of asymptotically aperiodic trajectories: Li and Yorke²⁰ entitle their original proof of this result "Period Three Implies Chaos".

The term "chaos" evokes an image of dynamical trajectories which are indistinguishable from some stochastic process. Numerical simulations^{12, 15, 21, 23, 25} of the dynamics of equation (3), (4) and other similar equations tend to confirm this impression. But, for smooth and "sensible" functions $F(X)$ such as in equations (3) and (4), the underlying mathematical fact is that for any specified parameter value there is one unique cycle that is stable, and that attracts essentially all initial points^{22, 26} (see ref. 4, appendix A, for a simple and lucid exposition). That is, there is one cycle that "owns" almost all initial points; the remaining infinite number of other cycles, along with the asymptotically aperiodic trajectories, own a set of points which, although uncountable, have measure zero.

As is made clear by Tables 3 and 4 below, any one particular stable cycle is likely to occupy an extraordinarily narrow window of parameter values. This fact, coupled with the long time it is likely to take for transients associated with the initial

conditions to damp out, means that in practice the unique cycle is unlikely to be unmasked, and that a stochastic description of the dynamics is likely to be appropriate, in spite of the underlying deterministic structure. This point is pursued further under the heading "practical applications", below.

The main messages of this section are summarised in Table 1, which sets out the various domains of dynamical behaviour of the equations (3) and (4) as functions of the parameters, a and r respectively, that determine the severity of the nonlinear response. These properties can be understood qualitatively in a graphical way, and are generic to any well behaved $F(X)$ in equation (1).

We now proceed to a more detailed discussion of the mathematical structure of the chaotic regime for analytical functions, and then to the practical problems alluded to above and to a consideration of the behavioural peculiarities exhibited by non-analytical functions (such as those in the two right hand columns of Table 1).

Fine structure of the chaotic regime

We have seen how the original fixed point X^* bifurcates to give harmonics of period 2^n . But how do new cycles of period k arise?

The general process is illustrated in Fig. 5, which shows how period 3 cycles originate. By an obvious extension of the notation introduced in equation (8), populations three generations apart are related by

$$X_{t+3} = F^{(3)}(X_t) \quad (11)$$

If the hump in $F(X)$ is sufficiently steep, the threefold iteration will produce a function $F^{(3)}(X)$ with 4 humps, as shown in Fig. 5 for the $F(X)$ of equation (3). At first (for $a < 3.8284 \dots$ in equation (3)) the 45° line intersects this curve only at the single point X^* (and at $X=0$), as shown by the solid curve in Fig. 5. As the hump in $F(X)$ steepens, the hills and valleys in $F^{(3)}(X)$ become more pronounced, until simultaneously the first two valleys sink and the final hill rises to touch the 45° line, and then to intercept it at 6 new points, as shown by the dashed curve in Fig. 5. These 6 points divide into two distinct three-point cycles. As can be made plausible by imagining the intermediate stages in Fig. 5, it can be shown that the stability-determining slope of $F^{(3)}(X)$ at three of these points has a common value, which is $\lambda^{(3)} = +1$ at their birth, and thereafter steepens beyond $+1$: this period 3 cycle is never stable. The slope of $F^{(3)}(X)$ at the other three points begins at $\lambda^{(3)} = +1$, and then decreases towards zero, resulting in a stable cycle of period 3. As $F(X)$ continues to steepen, the slope $\lambda^{(3)}$ for this initially stable three-point cycle decreases beyond -1 ; the cycle becomes unstable, and gives rise by the bifurcation process discussed in the previous section to stable cycles of period 6, 12, 24, $\dots$, 3×2^n . This birth of a stable and unstable pair of period 3 cycles, and the subsequent harmonics which arise as the initially stable cycle becomes unstable, are illustrated to the right of Fig. 4.

Table 1 Summary of the way various "single-hump" functions $F(X)$, from equation (1), behave in the chaotic region, distinguishing the dynamical properties which are generic from those which are not

The function $F(X)$ of equation (1)	$aX(1-X)$	$X \exp[r(1-X)]$	aX ; if $X < \frac{1}{2}$ $a(1-X)$; if $X > \frac{1}{2}$	λX ; if $X < 1$ λX^{1-b} ; if $X > 1$
Tunable parameter	a	r	a	b
Fixed point becomes unstable	3.0000	2.0000	1.0000*	2.0000
"Chaotic" region begins				
[point of accumulation of cycles of period 2^n]	3.5700	2.6924	1.0000	2.0000
First odd-period cycle appears	3.6786	2.8332	1.4142	2.6180
Cycle with period 3 appears				
[and therefore every integer period present]	3.8284	3.1024	1.6180	3.0000
"Chaotic" region ends	4.0000†	∞ ‡	2.000†	∞ ‡
Are there stable cycles in the chaotic region?	Yes	Yes	No	No

* Below this a value, $X = 0$ is stable.

† All solutions are attracted to $-\infty$ for a values beyond this.

‡ In practice, as r or b becomes large enough, X will eventually be carried so low as to be effectively zero, thus producing extinction in models of biological populations.

Table 2 Catalogue of the number of periodic points, and of the various cycles (with periods $k = 1$ up to 12), arising from equation (1) with a single-humped function $F(X)$

k	1	2	3	4	5	6	7	8	9	10	11	12
Possible total number of points with period k	2	4	8	16	32	64	128	256	512	1,024	2,048	4,096
Possible total number of points with non-degenerate period k	2	2	6	12	30	54	126	240	504	990	2,046	4,020
Total number of cycles of period k , including those which are degenerate and/or harmonics and/or never locally stable	2	3	4	6	8	14	20	36	60	108	188	352
Total number of non-degenerate cycles (including harmonics and unstable cycles)	2	1	2	3	6	9	18	30	56	99	186	335
Total number of non-degenerate, stable cycles (including harmonics)	1	1	1	2	3	5	9	16	28	51	93	170
Total number of non-degenerate, stable cycles whose basic period is k (that is, excluding harmonics)	1	—	1	1	3	4	9	14	28	48	93	165

There are, therefore, two basic kinds of bifurcation processes^{1,4} for first order difference equations. Truly new cycles of period k arise in pairs (one stable, one unstable) as the hills and valleys of higher iterates of $F(X)$ move, respectively, up and down to intercept the 45° line, as typified by Fig. 5. Such cycles are born at the moment when the hills and valleys become tangent to the 45° line, and the initial slope of the curve $F^{(k)}$ at the points is thus $\lambda^{(k)} = +1$: this type of bifurcation may be called^{1,4} a tangent bifurcation or a $\lambda = +1$ bifurcation. Conversely, an originally stable cycle of period k may become unstable as $F(X)$ steepens. This happens when the slope of $F^{(k)}$ at these period k points steepens beyond $\lambda^{(k)} = -1$, whereupon a new and initially stable cycle of period $2k$ is born in the way typified by Figs 2 and 3. This type of bifurcation may be called a pitchfork bifurcation (borrowing an image from the left hand side of Fig. 4) or a $\lambda = -1$ bifurcation^{1,4}.

Putting all this together, we conclude that as the parameters in $F(X)$ are varied the fundamental, stable dynamical units are cycles of basic period k , which arise by tangent bifurcation, along with their associated cascade of harmonics of periods $k2^n$, which arise by pitchfork bifurcation. On this basis, the constant equilibrium solution X^* and the subsequent hierarchy of stable cycles of periods 2^n is merely a special case, albeit a conspicuously important one (namely $k=1$), of a general phenomenon. In addition, remember^{1,4,22,29} that for sensible, analytical functions (such as, for example, those in equations (3) and (4)) there is a unique stable cycle for each value of the parameter in $F(X)$. The entire range of parameter values ($1 < a < 4$ in equation (3), $0 < r$ in equation (4)) may thus be regarded as made up of infinitely many windows of parameter

values—some large, some unimaginably small—each corresponding to a single one of these basic dynamical units. Tables 3 and 4, below, illustrate this notion. These windows are divided from each other by points (the points of accumulation of the harmonics of period $k2^n$) at which the system is truly chaotic, with no attractive cycle: although there are infinitely many such special parameter values, they have measure zero on the interval of all values.

How are these various cycles arranged along the interval of relevant parameter values? This question has to my knowledge been answered independently by at least 6 groups of people, who have seen the problem in the context of combinatorial theory^{18,30}, numerical analysis^{13,14}, population biology¹, and dynamical systems theory^{22,31} (broadly defined).

A simple-minded approach (which has the advantage of requiring little technical apparatus, and the disadvantage of being rather clumsy) consists of first answering the question, how many period k points can there be? That is, how many distinct solutions can there be to the equation

$$X^{*k} = F^{(k)}(X^{*k})? \quad (12)$$

If the function $F(X)$ is sufficiently steeply humped, as it will be once the parameter values are sufficiently large, each successive iteration doubles the number of humps, so that $F^{(k)}(X)$ has 2^{k-1} humps. For large enough parameter values, all these hills and valleys will intersect the 45° line, producing 2^k fixed points of period k . These are listed for $k \leq 12$ in the top row of Table 2. Such a list includes degenerate points of period k , whose period is a submultiple of k ; in particular, the two period 1 points ($X=0$ and X^*) are degenerate solutions of equation (12) for all k . By working from left to right across Table 2, these degenerate points can be subtracted out, to leave the total number of non-degenerate points of basic period k , as listed in the second row of Table 2. More sophisticated ways of arriving at this result are given elsewhere^{13,14,16,22,30,31}.

For example, there eventually are $2^6=64$ points with period 6. These include the two points of period 1, the period 2 "harmonic" cycle, and the stable and unstable pair of triplets of points with period 3, for a total of 10 points whose basic period is a submultiple of 6; this leaves 54 points whose basic period is 6.

The 2^k period k points are arranged into various cycles of period k , or submultiples thereof, which appear in succession by either tangent or pitchfork bifurcation as the parameters in $F(X)$ are varied. The third row in Table 2 catalogues the total number of distinct cycles of period k which so appear. In the fourth row¹⁴, the degenerate cycles are subtracted out, to give the total number of non-degenerate cycles of period k : these numbers must equal those of the second row divided by k . This fourth row includes the (stable) harmonics which arise by pitchfork bifurcation, and the pairs of stable-unstable cycles arising by tangent bifurcation. By subtracting out the cycles which are unstable from birth, the total number of possible stable cycles is given in row five; these figures can also be obtained by less pedestrian methods^{13,16,30}. Finally we may subtract out the stable cycles which arise by pitchfork bifurcation, as harmonics of some simpler cycle, to arrive at the final

Fig. 5 The relationship between X_{t+3} and X_t , obtained by three iterations of equation (3). The solid curve is for $a = 3.7$, and only intersects the 45° line once. As a increases, the hills and valleys become more pronounced. The dashed curve is for $a = 3.9$, and six new period 3 points have appeared (arranged as two cycles, each of period 3).

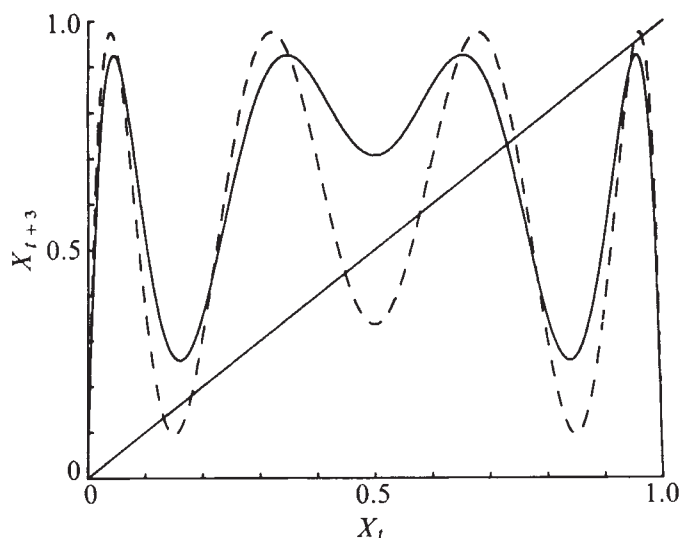


Table 3 A catalogue of the stable cycles (with basic periods up to 6) for the equation $X_{t+1} = aX_t(1 - X_t)$

Period of basic cycle	a value at which:		Subsequent cascade of "harmonics" with period $k2^n$ all become unstable	Width of the range of a values over which the basic cycle, or one of its harmonics, is attractive
	Basic cycle first appears	Basic cycle becomes unstable		
1	1.0000	3.0000	3.5700	2.5700
3	3.8284	3.8415	3.8495	0.0211
4	3.9601	3.9608	3.9612	0.0011
5(a)	3.7382	3.7411	3.7430	0.0048
5(b)	3.9056	3.9061	3.9065	0.0009
5(c)	3.99026	3.99030	3.99032	0.00006
6(a)	3.6265	3.6304	3.6327	0.0062
6(b)	3.937516	3.937596	3.937649	0.000133
6(c)	3.977760	3.977784	3.977800	0.000040
6(d)	3.997583	3.997585	3.997586	0.000003

row in Table 2, which lists the number of stable cycles whose basic period is k .

Returning to the example of period 6, we have already noted the five degenerate cycles whose periods are submultiples of 6. The remaining 54 points are parcelled out into one cycle of period 6 which arises as the harmonic of the only stable three-point cycle, and four distinct pairs of period 6 cycles (that is, four initially stable ones and four unstable ones) which arise by successive tangent bifurcations. Thus, reading from the foot of the column for period 6 in Table 2, we get the numbers 4, 5, 9, 14.

Using various labelling tricks, or techniques from combinatorial theory, it is also possible to give a generic list of the order in which the various cycles appear^{1,13,16,22}. For example, the basic stable cycles of periods 3, 5, 6 (of which there are respectively 1, 3, 4) must appear in the order 6, 5, 3, 5, 6, 6, 5, 6: compare Tables 3 and 4. Metropolis *et al.*¹⁶ give the explicit such generic list for all cycles of period $k \leq 11$.

As a corollary it follows that, given the most recent cycle to appear, it is possible (at least in principle) to catalogue all the cycles which have appeared up to this point. An especially elegant way of doing this is given by Smale and Williams²², who show, for example, that when the stable cycle of period 3 first originates, the total number of other points with periods k , N_k , which have appeared by this stage satisfy the Fibonacci series, $N_k = 2, 4, 5, 8, 12, 19, 30, 48, 77, 124, 200, 323$ for $k = 1, 2, \dots, 12$: this is to be contrasted with the total number of points of period k which will eventually appear (the top row of Table 2) as $F(X)$ continues to steepen.

Such catalogues of the total number of fixed points, and of their order of appearance, are relatively easy to construct. For any particular function $F(X)$, the numerical task of finding the windows of parameter values wherein any one cycle or its harmonics is stable is, in contrast, relatively tedious and inelegant. Before giving such results, two critical parameter values of special significance should be mentioned.

Hoppensteadt and Hyman²¹ have given a simple graphical method for locating the parameter value in the chaotic regime at which the first odd period cycle appears. Their analytic recipe is as follows. Let α be the parameter which tunes the steepness of $F(X)$ (for example, $\alpha = a$ for equation (3), $\alpha = r$ for equation (4)), $X^*(\alpha)$ be the fixed point of period 1 (the non-trivial solution of equation (5)), and $X_{max}(\alpha)$ the maximum value attainable from iterations of equation (1) (that is, the value of $F(X)$ at its hump or stationary point). The first odd period cycle appears for that value of α which satisfies^{21,31}

$$X^*(\alpha) = F^{(2)}(X_{max}(\alpha)) \quad (13)$$

As mentioned above, another critical value is that where the period 3 cycle first appears. This parameter value may be found numerically from the solutions of the third iterate of equation (1): for equation (3) it is¹⁴ $a = 1 + \sqrt{8}$.

Myrberg¹³ (for all $k \leq 10$) and Metropolis *et al.*¹⁶ (for all $k \leq 7$) have given numerical information about the stable cycles in equation (3). They do not give the windows of parameter

values, but only the single value at which a given cycle is maximally stable; that is, the value of a for which the stability-determining slope of $F^{(k)}(X)$ is zero, $\lambda^{(k)} = 0$. Since the slope of the k -times iterated map $F^{(k)}$ at any point on a period k cycle is simply equal to the product of the slopes of $F(X)$ at each of the points X^*_k on this cycle^{1,8,20}, the requirement $\lambda^{(k)} = 0$ implies that $X = A$ (the stationary point of $F(X)$, where $\lambda^{(1)} = 0$) is one of the periodic points in question, which considerably simplifies the numerical calculations.

For each basic cycle of period k (as catalogued in the last row of Table 2), it is more interesting to know the parameter values at which: (1) the cycle first appears (by tangent bifurcation); (2) the basic cycle becomes unstable (giving rise by successive pitchfork bifurcations to a cascade of harmonics of periods $k2^n$); (3) all the harmonics become unstable (the point of accumulation of the period $k2^n$ cycles). Tables 3 and 4 extend the work of May and Oster¹, to give this numerical information for equations (3) and (4), respectively. (The points of accumulation are not ground out mindlessly, but are calculated by a rapidly convergent iterative procedure, see ref. 1, appendix A.) Some of these results have also been obtained by Gumowski and Mira²².

Practical problems

Referring to the paradigmatic example of equation (3), we can now see that the parameter interval $1 < a < 4$ is made up of a one-dimensional mosaic of infinitely many windows of a -values, in each of which a unique cycle of period k , or one of its harmonics, attracts essentially all initial points. Of these windows, that for $1 < a < 3.5700 \dots$ corresponding to $k=1$ and its harmonics is by far the widest and most conspicuous. Beyond the first point of accumulation, it can be seen from Table 3 that these windows are narrow, even for cycles of quite low periods, and the windows rapidly become very tiny as k increases.

As a result, there develops a dichotomy between the underlying mathematical behaviour (which is exactly determinable) and the "commonsense" conclusions that one would draw from numerical simulations. If the parameter a is held constant at one value in the chaotic region, and equation (3) iterated for an arbitrarily large number of generations, a density plot of the observed values of X_t on the interval 0 to 1 will settle into k equal spikes (more precisely, delta functions) corresponding to the k points on the stable cycle appropriate to this a -value. But for most a -values this cycle will have a fairly large period, and moreover it will typically take many thousands of generations before the transients associated with the initial conditions are damped out: thus the density plot produced by numerical simulations usually looks like a sample of points taken from some continuous distribution.

An especially interesting set of numerical computations are due to Hoppensteadt (personal communication) who has combined many iterations to produce a density plot of X_t for each one of a sequence of a -values, gradually increasing from 3.5700 $\dots$ to 4. These results are displayed as a movie. As can be expected from Table 3, some of the more conspicuous cycles

do show up as sets of delta functions: the 3-cycle and its first few harmonics; the first 5-cycle; the first 6-cycle. But for most values of a the density plot looks like the sample function of a random process. This is particularly true in the neighbourhood of the a -value where the first odd cycle appears ($a=3.6786 \dots$), and again in the neighbourhood of $a=4$: this is not surprising, because each of these locations is a point of accumulation of points of accumulation. Despite the underlying discontinuous changes in the periodicities of the stable cycles, the observed density pattern tends to vary smoothly. For example, as a increases toward the value at which the 3-cycle appears, the density plot tends to concentrate around three points, and it smoothly diffuses away from these three points after the 3-cycle and all its harmonics become unstable.

I think the most interesting mathematical problem lies in designing a way to construct some approximate and "effectively continuous" density spectrum, despite the fact that the exact density function is determinable and is always a set of delta functions. Perhaps such techniques have already been developed in ergodic theory³³ (which lies at the foundations of statistical mechanics), as for example in the use of "coarse-grained observers". I do not know.

Such an effectively stochastic description of the dynamical properties of equation (4) for large r has been provided²⁸, albeit by tactical tricks peculiar to that equation rather than by any general method. As r increases beyond about 3, the trajectories generated by this equation are, to an increasingly good approximation, almost periodic with period $(1/r) \exp(r-1)$.

The opinion I am airing in this section is that although the exquisite fine structure of the chaotic regime is mathematically fascinating, it is irrelevant for most practical purposes. What seems called for is some effectively stochastic description of the deterministic dynamics. Whereas the various statements about the different cycles and their order of appearance can be made in generic fashion, such stochastic description of the actual dynamics will be quite different for different $F(X)$: witness the difference between the behaviour of equation (4), which for large r is almost periodic "outbreaks" spaced many generations apart, versus the behaviour of equation (3), which for $a \rightarrow 4$ is not very different from a series of Bernoulli coin flips.

Mathematical curiosities

As discussed above, the essential reason for the existence of a succession of stable cycles throughout the "chaotic" regime is that as each new pair of cycles is born by tangent bifurcation (see Fig. 5), one of them is at first stable, by virtue of the way the smoothly rounded hills and valleys intercept the 45° line. For analytical functions $F(X)$, the only parameter values for which the density plot or "invariant measure" is continuous and truly ergodic are at the points of accumulation of harmonics, which divide one stable cycle from the next. Such exceptional parameter values have found applications, for example, in the use of equation (3) with $a=4$ as a random number generator^{34,35}: it has a continuous density function proportional to $[X(1-X)]^{-1}$ in the interval $0 < X < 1$.

Non-analytical functions $F(X)$ in which the hump is in fact a spike provide an interesting special case. Here we may imagine spikey hills and valleys moving to intercept the 45° line in Fig. 5, and it may be that both the cycles born by tangent bifurcation are unstable from the outset (one having $\lambda^{(k)} > 1$, the other $\lambda^{(k)} < -1$), for all $k > 1$. There are then no stable cycles in the chaotic regime, which is therefore literally chaotic with a continuous and truly ergodic density distribution function.

One simple example is provided by

$$\begin{aligned} X_{t+1} &= aX_t; \text{ if } X_t < \frac{1}{2} \\ X_{t+1} &= a(1-X_t); \text{ if } X_t > \frac{1}{2} \end{aligned} \quad (14)$$

defined on the interval $0 < X < 1$. For $0 < a < 1$, all trajectories are attracted to $X=0$; for $1 < a < 2$, there are infinitely many periodic orbits, along with an uncountable number of aperiodic trajectories, none of which are locally stable. The first odd period cycle appears at $a=\sqrt{2}$, and all integer periods are represented beyond $a=(1+\sqrt{5})/2$. Kac³⁶ has given a careful discussion of the case $a=2$. Another example, this time with an extensive biological pedigree¹⁻³, is the equation

$$\begin{aligned} X_{t+1} &= \lambda X_t; \text{ if } X_t < 1 \\ X_{t+1} &= \lambda X_t^{1-b}; \text{ if } X_t > 1 \end{aligned} \quad (15)$$

If $\lambda > 1$ this possesses a globally stable equilibrium point for $b < 2$. For $b > 2$ there is again true chaos, with no stable cycles: the first odd cycle appears at $b=(3+\sqrt{5})/2$, and all integer periods are present beyond $b=3$. The dynamical properties of equations (14) and (15) are summarised to the right of Table 2.

The absence of analyticity is a necessary, but not a sufficient, condition for truly random behaviour³¹. Consider, for example,

$$\begin{aligned} X_{t+1} &= (a/2)X_t; \text{ if } X_t < \frac{1}{2} \\ X_{t+1} &= aX_t(1-X_t); \text{ if } X_t > \frac{1}{2} \end{aligned} \quad (16)$$

This is the parabola of equation (3) and Fig. 1, but with the left hand half of $F(X)$ flattened into a straight line. This equation does possess windows of a values, each with its own stable cycle, as described generically above. The stability-determining slopes $\lambda^{(k)}$ vary, however, discontinuously with the parameter a , and the widths of the simpler stable regions are narrower than for equation (3): the fixed point becomes unstable at $a=3$; the point of accumulation of the subsequent harmonics is at $a=3.27 \dots$; the first odd cycle appears at $a=3.44 \dots$; the 3-point cycle at $a=3.67 \dots$ (compare the first column in Table 1).

These eccentricities of behaviour manifested by non-analytical functions may be of interest for exploring formal questions in ergodic theory. I think, however, that they have no relevance to models in the biological and social sciences, where functions such as $F(X)$ should be analytical. This view is elaborated elsewhere³⁷.

As a final curiosity, consider the equation

$$X_{t+1} = \lambda X_t [1 + X_t]^{-b} \quad (17)$$

Table 4 Catalogue of the stable cycles (with basic periods up to 6) for the equation $X_{t+1} = X_t \exp[r(1-X_t)]$

Period of basic cycle	r value at which:		Subsequent cascade of "harmonics" with period $k2^n$ all become unstable	Width of the range of r values over which the basic cycle, or one of its harmonics, is attractive
	Basic cycle first appears	Basic cycle becomes unstable		
1	0.0000	2.0000	2.6924	2.6924
3	3.1024	3.1596	3.1957	0.0933
4	3.5855	3.6043	3.6153	0.0298
5(a)	2.9161	2.9222	2.9256	0.0095
5(b)	3.3632	3.3664	3.3682	0.0050
5(c)	3.9206	3.9295	3.9347	0.0141
6(a)	2.7714	2.7761	2.7789	0.0075
6(b)	3.4558	3.4563	3.4567	0.0009
6(c)	3.7736	3.7745	3.7750	0.0014
6(d)	4.1797	4.1848	4.1880	0.0083

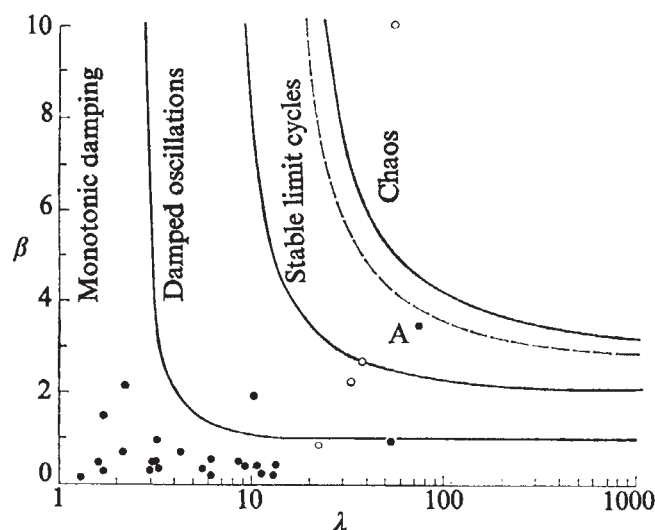


Fig. 6 The solid lines demarcate the stability domains for the density dependence parameter, β , and the population growth rate, λ , in equation (17); the dashed line shows where 2-point cycles give way to higher cycles of period 2^n . The solid circles come from analyses of life table data on field populations, and the open circles from laboratory populations (from ref. 3, after ref. 39).

This has been used to fit a considerable amount of data on insect populations^{38,39}. Its stability behaviour, as a function of the two parameters λ and β , is illustrated in Fig. 6. Notice that for $\lambda < 7.39$... there is a globally stable equilibrium point for all β ; for $7.39 < \lambda < 12.50$... this fixed point becomes unstable for sufficiently large β , bifurcating to a stable 2-point cycle which is the solution for all larger β ; as λ increases through the range $12.50 < \lambda < 14.77$... various other harmonics of period 2^n appear in turn. The hierarchy of bifurcating cycles of period 2^n is thus truncated, and the point of accumulation and subsequent regime of chaos is not achieved (even for arbitrarily large β) until $\lambda > 14.77$...

Applications

The fact that the simple and deterministic equation (1) can possess dynamical trajectories which look like some sort of random noise has disturbing practical implications. It means, for example, that apparently erratic fluctuations in the census data for an animal population need not necessarily betoken either the vagaries of an unpredictable environment or sampling errors: they may simply derive from a rigidly deterministic population growth relationship such as equation (1). This point is discussed more fully and carefully elsewhere¹.

Alternatively, it may be observed that in the chaotic regime arbitrarily close initial conditions can lead to trajectories which, after a sufficiently long time, diverge widely. This means that, even if we have a simple model in which all the parameters are determined exactly, long term prediction is nevertheless impossible. In a meteorological context, Lorenz¹⁶ has called this general phenomenon the "butterfly effect": even if the atmosphere could be described by a deterministic model in which all parameters were known, the fluttering of a butterfly's wings could alter the initial conditions, and thus (in the chaotic regime) alter the long term prediction.

Fluid turbulence provides a classic example where, as a parameter (the Reynolds number) is tuned in a set of deterministic equations (the Navier-Stokes equations), the motion can undergo an abrupt transition from some stable configuration (for example, laminar flow) into an apparently stochastic, chaotic regime. Various models, based on the Navier-Stokes differential equations, have been proposed as mathematical metaphors for this process^{15,40,41}. In a recent review of the theory of turbulence, Martin⁴² has observed that the one-

dimensional difference equation (1) may be useful in this context. Compared with the earlier models^{15,40,41}, it has the disadvantage of being even more abstractly metaphorical, and the advantage of having a spectrum of dynamical behaviour which is more richly complicated yet more amenable to analytical investigation.

A more down-to-earth application is possible in the use of equation (1) to fit data^{1,2,3,38,39,43} on biological populations with discrete, non-overlapping generations, as is the case for many temperate zone arthropods. Figure 6 shows the parameter values λ and β that are estimated³⁹ for 24 natural populations and 4 laboratory populations when equation (17) is fitted to the available data. The figure also shows the theoretical stability domains: a stable point; its stable harmonics (stable cycles of period 2^n); chaos. The natural populations tend to have stable equilibrium point behaviour. The laboratory populations tend to show oscillatory or chaotic behaviour; their behaviour may be exaggeratedly nonlinear because of the absence, in a laboratory setting, of many natural mortality factors. It is perhaps suggestive that the most oscillatory natural population (labelled A in Fig. 6) is the Colorado potato beetle, whose present relationship with its host plant lacks an evolutionary pedigree. These remarks are only tentative, and must be treated with caution for several reasons. Two of the main caveats are that there are technical difficulties in selecting and reducing the data, and that there are no single species populations in the natural world: to obtain a one-dimensional difference equation by replacing a population's interactions with its biological and physical environment by passive parameters (such as λ and β) may do great violence to the reality.

Some of the many other areas where these ideas have found applications were alluded to in the second section, above⁵⁻¹¹. One aim of this review article is to provoke applications in yet other fields.

Related phenomena in higher dimensions

Pairs of coupled, first-order difference equations (equivalent to a single second-order equation) have been investigated in several contexts^{4,44-46}, particularly in the study of temperate zone arthropod prey-predator systems^{2-4,23,47}. In these two-dimensional systems, the complications in the dynamical behaviour are further compounded by such facts as: (1) even for analytical functions, there can be truly chaotic behaviour (as for equations (14) and (15)), corresponding to so-called "strange attractors"; and (2) two or more different stable states (for example, a stable point and a stable cycle of period 3) can occur together for the same parameter values⁴. In addition, the manifestation of these phenomena usually requires less severe nonlinearities (less steeply humped $F(X)$) than for the one-dimensional case.

Similar systems of first-order ordinary differential equations, or two coupled first-order differential equations, have much simpler dynamical behaviour, made up of stable and unstable points and limit cycles⁴⁸. This is basically because in continuous two-dimensional systems the inside and outside of closed curves can be distinguished; dynamic trajectories cannot cross each other. The situation becomes qualitatively more complicated, and in many ways analogous to first-order difference equations, when one moves to systems of three or more coupled, first-order ordinary differential equations (that is, three-dimensional systems of ordinary differential equations). Scanlon (personal communication) has argued that chaotic behaviour and "strange attractors", that is solutions which are neither points nor periodic orbits⁴⁸, are typical of such systems. Some well studied examples arise in models for reaction-diffusion systems in chemistry and biology⁴⁹, and in the models of Lorenz¹⁵ (three dimensions) and Ruelle and Takens⁴⁰ (four dimensions) referred to above. The analysis of these systems is, by virtue of their higher dimensionality, much less transparent than for equation (1).

An explicit and rather surprising example of a system which

has recently been studied from this viewpoint is the ordinary differential equations used in ecology to describe competing species. For one or two species these systems are very tame: dynamic trajectories will converge on some stable equilibrium point (which may represent coexistence, or one or both species becoming extinct). As Smale⁵⁰ has recently shown, however, for 3 or more species these general equations can, in a certain reasonable and well-defined sense, be compatible with any dynamical behaviour. Smale's⁵⁰ discussion is generic and abstract: a specific study of the very peculiar dynamics which can be exhibited by the familiar Lotka-Volterra equations once there are 3 competitors is given by May and Leonard⁵¹.

Conclusion

In spite of the practical problems which remain to be solved, the ideas developed in this review have obvious applications in many areas.

The most important applications, however, may be pedagogical.

The elegant body of mathematical theory pertaining to linear systems (Fourier analysis, orthogonal functions, and so on), and its successful application to many fundamentally linear problems in the physical sciences, tends to dominate even moderately advanced University courses in mathematics and theoretical physics. The mathematical intuition so developed ill equips the student to confront the bizarre behaviour exhibited by the simplest of discrete nonlinear systems, such as equation (3). Yet such nonlinear systems are surely the rule, not the exception, outside the physical sciences.

I would therefore urge that people be introduced to, say, equation (3) early in their mathematical education. This equation can be studied phenomenologically by iterating it on a calculator, or even by hand. Its study does not involve as much conceptual sophistication as does elementary calculus. Such study would greatly enrich the student's intuition about nonlinear systems.

Not only in research, but also in the everyday world of politics and economics, we would all be better off if more people realised that simple nonlinear systems do not necessarily possess simple dynamical properties.

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articles

Prenatal genesis of connections subserving ocular dominance in the rhesus monkey

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In foetal monkey brain neuronal projections carrying input from the two eyes initially overlap; they segregate during the second half of gestation and become fully separated in subcortical visual centres and partially separated in the cortex three weeks before birth and thus before visual experience.

DEVELOPMENT of the binocular visual system has been a major topic of research since the demonstration more than a decade ago that neuronal function can be altered in kittens deprived of vision in one eye¹. More recently, similar observations in the rhesus monkey² have underlined the relevance of this research to an understanding of mechanisms of amblyopia ex anopsia in children. In several respects, the development of the visual system is similar in

Table 1 Experimental protocol for three foetal monkeys injected at various times for autoradiography

Animal	Embryonic day (E) when injected	Embryonic day (E) when killed	Exposure time for LGd and SCsg (weeks)	Exposure time for cortex (weeks)
(1) 052775	E64	E78	2, 4	2, 4, 8, 16, 20, 24
(2) 021075	E110	E124	2, 4	2, 4, 8, 16, 20, 24
(3) 061275	E130	E144	2, 4	2, 4, 8, 16, 20, 24

Three foetal monkeys were injected once each at the specified embryonic (E) day into one eye with equal parts of ^3H -proline and ^3H -fucose. After injection, each foetus was replaced in the uterus and killed 14 d later.

man and monkey. For example, in both species all neurones that comprise the visual system are generated during the first two-thirds of gestation²⁻⁵. In both species, approximately one-half of the axons from the retinal ganglion cells of each eye are distributed at the optic chiasma to both the ipsilateral and the contralateral dorsal lateral geniculate bodies (LGd). In both man and monkey, the LGd has 6 laminae. Three laminae (1, 4 and 6) receive direct inputs from the contralateral eye and the remaining three (2, 3 and 5) from the ipsilateral eye⁶.

The recently developed autoradiographic method of orthograde axoplasmic⁷ and transneuronal transport^{8,9} provide a simple and powerful method for exploring the structural development of the binocular visual system by tracing the distribution of radioactive label following injection of isotopes into one eye. Radioactivity transported to the appropriate laminae of the LGd and transneuronal to the primary visual cortex (area 17 of Brodmann) is distributed in the sublayers IVA and IVC over columns 350 μm wide that alternate with columns of the same width containing low grain counts⁹. The autoradiographic method thus confirms earlier more traditional anatomical and

electrophysiological evidence for columnar organisation. It has also provided the first anatomical evidence for a segregated representation of each eye in the superior colliculus (SC), where fibres from the two eyes terminate in complementary, alternating territories 0.1–0.5 mm wide^{10,11}.

Autoradiography has also been used to determine the anatomical consequences of monocular deprivation at early postnatal ages. This method reveals a reciprocal expansion and contraction of the ocular dominance columns in layer IV of the visual cortex that are related to non-occluded and occluded eyes, respectively¹². It has been suggested that the basic connections subserving binocular vision are determined innately, even though, as mentioned above, changes in the width of columns can be induced by an imbalance in visual experience during the first few postnatal months¹². This interpretation, however, has not been fully accepted by some investigators who argue that visual experience rather than genetic factors may play a fundamental inductive role in establishing functional connections. (For a recent review on the subject, see ref. 13.) An analysis of the prenatal development of the circuitry involved in ocular dominance in the monkey may therefore help to resolve the issue.

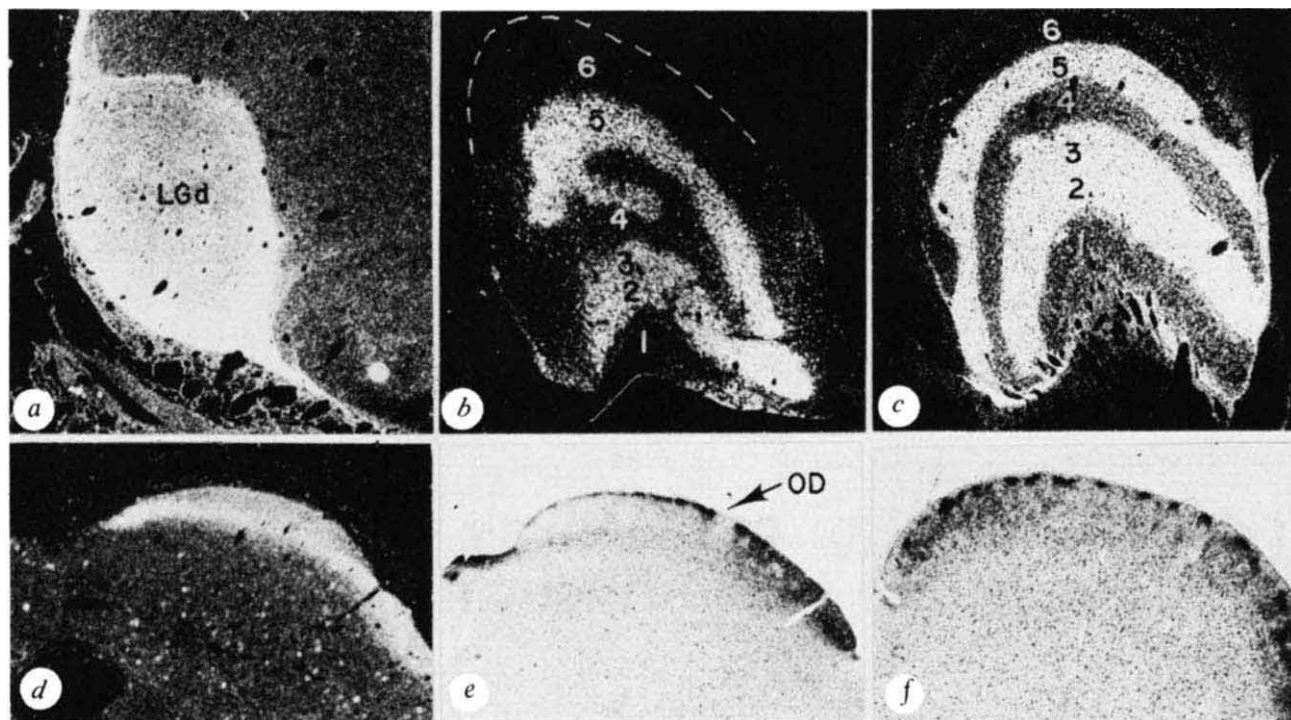


Fig. 1 Dark-field and bright-field illumination photographs of autoradiograms from foetuses exposed by hysterotomy and injected into the vitreous body of one eye with a mixture of ^3H -proline and ^3H -fucose at different embryonic (E) days. Foetuses were replaced in the uterus and killed 14 d later by a second caesarian section. All photographs are the same magnification ($\times 20$). The terms "contralateral" and "ipsilateral" are used with respect to the injected eye. *a*, Contralateral LGd in the foetus injected at E64 and killed at E78. The radioactive label does not suggest a laminar pattern. *b*, Ipsilateral LGd in the foetus injected with comparable doses of the same isotopes at E110, killed at E124. Label is concentrated over the emerging laminae 2, 3 and 5. *c*, Dark-field photograph of a coronal section of the ipsilateral superior colliculus (SC) of the foetus injected at E64 and killed at E78. Label is uniformly distributed over the entire nucleus. *d*, Bright-field photograph of a sagittal section of the contralateral SC in the foetus injected at E110 and killed at E124. Retinotectal projections are clearly segregated into ocular dominance patches indicated by alternating areas of high and low grain counts. The large, empty zone corresponds to the optic disk (OD) representation. *e*, Bright-field photograph of a coronal section of the ipsilateral SC in the foetus injected and killed at slightly later gestational ages (E130–E144). Retinotectal projections are concentrated into discrete patches of dense labelling that correspond to areas devoid of grains in the contralateral SC. The optic disk representation is not present at this level.

Pregnant rhesus monkeys (*Macaca mulatta*) were subjected to laparotomy and hysterotomy under halothane-oxygen anaesthesia at appropriate gestational intervals (Table 1). Equal amounts of ^3H -proline and ^3H -fucose (total 1.0–1.5 mCi) were injected into the vitreous body of one eye of each foetus. After injection, the foetuses were returned to the uterus, and the chorio-allantoic membrane, uterine and abdominal walls were sutured in layers. Fourteen days later, after an interval sufficient for concentration of tracer in LGd, SC and visual cortex by axonal and transneuronal transport, the living foetuses were removed from the uterus by a second caesarian section and perfused intracardially with a buffered paraformaldehyde-glutaraldehyde mixture. The brains were embedded in polyester wax, sectioned serially at 8 μm and processed by the standard autoradiographic method.

Retinogeniculate projections

The youngest foetus in this series was injected at E64 (embryonic day 64) by which time all LGd neurones have been generated¹⁴. This foetus was killed at E78, before the LGd becomes laminated³. Thus, this experiment was designed to determine whether retinal ganglion cell axons from each eye are segregated in the LGd before it develops laminae.

Radioactive tracer, transported in an orthograde direction from ganglion cells in the injected eye to the optic nerve, through the optic chiasma and the optic tracts, is concentrated in approximately equal amounts in both LGds. Evidently, equal numbers of crossed and uncrossed retinal fibres are already distributed to each LGd at this foetal age. Although there are areas with slightly higher or lower grain densities, segregation into separate layers is not discernible (Fig. 1a). Rather, grains are distributed more or less diffusely throughout the entire ipsilateral and contralateral LGd, indicating the possibility that at an early stage of development (around E64) projections from both eyes basically overlap in their distribution among neurones of the LGd with no evidence of the laminar segregation characteristically seen in the adult nucleus.

The second foetus in this series was injected in one eye with a mixture of ^3H -proline and ^3H -fucose at E110, that is, at an age when the characteristic laminar pattern of the LGd is beginning to emerge³. Fourteen days later, at E124, when this foetus was killed, the six-layered pattern of cells of the LGd, although still somewhat irregular, is already clearly developed. The laminae 2, 3 and 5 of the LGd are found to be intensely labelled ipsilaterally to the injected eye (Fig. 1b) whereas laminae 1, 4 and 6 are labelled in the contralateral nucleus. The laminar segregation of ipsilateral and contralateral retinal inputs to LGd therefore occurs in the interval between E64 and E110 when laminar segregation of their target cells is also achieved. In both LGd, the more ventral laminae (one contralaterally and two ipsilaterally) are more intensely labelled than the more dorsal ones.

The distribution of radioactive label in a third foetus injected and killed at slightly later gestational ages (E130–E144) is essentially the same, except that the distribution of grains over the appropriate laminae resembles closely the adult pattern (Fig. 1c); that is, the laminar organisation of cells in the LGd is more regular and more sharply outlined than seen in younger foetuses.

Retinotectal projections

Substantial amounts of radioactivity were transported through the brachium of the superior colliculus into the tectum in all three foetuses. In the youngest foetus, which was injected at E64 and killed at E78, the tracer is concentrated uniformly over the superficial layer of both SC with no evidence of complementary segregation of projections from the ipsilateral and contralateral eyes (Fig. 1d). The amount of label is noticeably greater on the contralateral

side, however. Fibres from the two eyes therefore initially overlap in the SC, as well as in the LGd.

By contrast, in the foetus injected at E110 and killed at E124, the radioactive label is distributed in a pattern of alternating dense and light concentrations (Fig. 1e). Although not as sharply defined, these correspond closely in number and pattern to the ocular dominance 'clumps' observed in the mature monkey¹¹. The alternating pattern is most evident in the posterior region of the SC where the retinal axons penetrate more deeply into the superficial grey. The width of the 'clumps' in the foetal monkey, ranging from 0.07 to 0.3 mm, is substantially smaller than that of the adult monkey (0.1–0.5 mm)¹¹, presumably reflecting the smaller size of the SC in the foetus. As in the adult, the pattern of grain distribution on both sides fits like pieces in a puzzle; that is, territories of the contralateral SC which contain low concentrations correspond to territories of the ipsilateral SC which contain high concentrations. In addition, a sector which contains no label in the contralateral SC represents the position of the optic disk at this foetal age (OD in Fig. 1e).

The oldest foetus in this study, injected at E130 and killed at E144, shows essentially the same pattern of alternating grain distribution in the SC. The contrast between heavily and lightly labelled territories is more distinct and the boundaries of these territories are defined as sharply as in the adult monkey (Fig. 1f).

Geniculocortical projections

Radioactive label was transported transneuronally into neurones of the LGd and the geniculocortical fibres of the three foetuses as revealed by a high concentration of label of the optic radiations (OR in Fig. 2a). Because the amount of radioactivity transferred transneuronally is less than 3% of that transported through the primary pathway⁸, autoradiographic exposures were lengthened appropriately for demonstration of geniculocortical projections (Table 1). As expected, this was done at the expense of relatively low background (Fig. 2).

The youngest foetus was injected at E64 and killed at E78, that is when only a fraction of neurones destined for layer IV of the visual cortex are generated³, and many of these neurones which have already undergone their last cell division have not yet reached their final position and are still migrating towards the cortex through the fibre plexus of the intermediate zone⁴. The autoradiograms show that fibres from the optic radiation (OR in Fig. 2a) have reached the occipital lobe, but they have not entered the developing cortex in any substantial number, and most of the label is concentrated in the subcortical intermediate zone of the developing cerebral wall. There is an abrupt decline in grain density at the external border of this zone (SP in Fig. 2a) which is situated external to the optic radiation but internal to the developing cortex. The distribution of grains in the intermediate zone forms a continuous, uniform band without periodic variations. Thus, at this stage in the telencephalon, there is no evidence of segregation of fibres derived from the two eyes. This is not unexpected since, in the same foetus, axons of the retinal projections from the two eyes are not yet segregated in the LGd (see above).

The second foetus was injected at E110 and was killed at E124, that is, after all cortical neurones are generated and also after they have completed their migration to the cortex⁴. In this foetus, the location of LGd axons are clearly identified by a dense concentration of grains over the optic radiation (OR) in the occipital lobe (Fig. 2b). Numerous fibres pass from the optic radiation towards the cortex of area 17, but stop abruptly at the border of this field with area 18 (Fig. 2b). Although the great majority of these fibres is concentrated in the intermediate zone

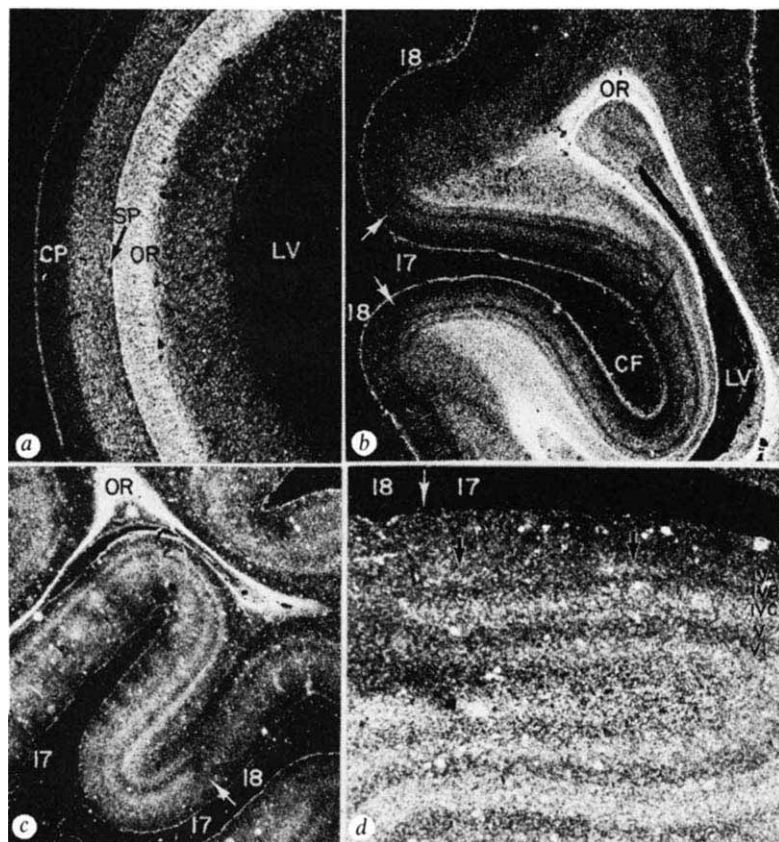


Fig. 2 Dark-field photographs of the cerebral wall and cortex at the level of the occipital lobe in the foetus injected with a mixture of ^3H -proline and ^3H -fucose at various foetal ages and killed 14 d later. The high grain background in these autoradiograms is due in part to the long exposure (Table 1) and partly to the high dose of ^3H -proline- ^3H -fucose mixture that produces unspecific labelling similar to background levels found in autoradiograms of liver, kidney, spleen and other organs prepared from the same foetuses. Therefore, only a concentration of grains well above background in the autoradiograms of the cerebrum is considered to represent radioactivity in the axons of the visual pathways. *a*, A coronal cut across the cerebral wall in the foetus injected at E64 and killed at E78. The large lateral vesicle (LV) is on the right. The optic radiation (OR) is heavily labelled, but fibres stop at the subcortical zone (SP) and do not invade the developing cortical plate (CP). Approximately $\times 27$. *b*, Occipital lobe of the foetus whose one eye was injected with the same mixture of labels at E110 and killed at E124. The optic radiation (OR) surrounds the lateral ventricle (LV) and emanates axons only to area 17 of the calcarine fissure (CF). Projections to the cortex stop sharply at the borderline between area 17 and area 18 (arrows). Some axons and/or terminals have entered the cortical plate but are distributed uniformly over layers IV and VI. Approximately $\times 8.7$. *c*, Visual cortex of the foetus injected and killed at a slightly older age (E130–E144). More axons and/or terminals have invaded the cortex and are concentrated over layers IV and VI in area 17. $\times 12$. *d*, Higher magnification photograph of the visual cortex demonstrates segregation of axons and/or terminals over sublayers IVA and IVC, as well as alternating regions of higher (black arrowheads) and lower grain counts. The limits of zones with high grain densities in both layers occur sharply at the borderline to area 18. $\times 30$.

below the primary visual area, small numbers of them do enter the cortex, where they are uniformly distributed over layers IV and VI (Fig. 2*b*). There is, however, no horizontal bilaminar concentration of silver grains over sublayers IVA and IVC and no alternating radially aligned concentrations of grains that might indicate the incipient formation of ocular dominance columns.

In the third foetus that was injected and killed at a slightly later age (E130–E144) more grains are concentrated over layers IV and VI presumably because more geniculocortical fibres have invaded the cortex. In this specimen, a bilaminar pattern of grain concentration can be discerned over sublayers IVA and IVC in autoradiograms exposed 12 to 24 weeks (Fig. 2*c*). The boundaries between these two sublayers are not as sharp as in the adult, however. By and large, the grains over sublayers IVA and IVC at this foetal age are distributed as a continuous sheet, although there are sectors in which an alternating light and dense pattern of grain concentration seems to emerge (Fig. 2*c*). This pattern is of low contrast and, although difficult to see in photomicrographs, it is clearly present when a grain count is performed (Fig. 3). Sectors with higher counts in sublayers IVA are in register with those of IVC, and the same is true of sectors with lower counts (Figs 2*c* and 3). In all likelihood, these represent the emerging ocular dominance columns. Presumably, only the sectors, 100–150 μm wide, which contain the highest grain counts (underlined numerals in Fig. 3) and those with the lowest grain counts (numerals between outlines) correspond to territories occupied predominantly by terminals derived from the injected and uninjected eye, respectively. Therefore, the width of the sectors with high concentration of grains is not 50% of the double columns (Fig. 3) which indicates that there is still considerable overlap between the terminals derived from the two eyes. The combined width of an ipsilateral and contralateral ocular dominance column as determined by the distance between two peaks in grain concentrations

is 500–600 μm . The comparable figure for the width of two columns in the mature monkey is 700–800 μm (ref. 9). The difference in width may be explained by a substantial increase in the cortical surface area between E144 and maturity.

Sequence of developmental events

The topographical interrelationship of the central connections in the adult visual system is the result of multiple, complex cellular interactions and events which occur during ontogeny. During the extended prenatal period of primates, young neurones change their position relative to one another; many of them migrate long distances to attain their final locations; and their dendritic and axonal processes differentiate and are deployed while the entire brain changes its external and internal form dramatically^{4,5,14}. These cellular activities are coordinated in space and in time and may play a fundamental role in the formation of complex cytoarchitectonic patterns and point-to-point connectivity of widely separated visual centres.

These observations indicate that cellular events fundamental to the prenatal development of the visual system in the rhesus monkey may be separated into two broad phases: in the first phase, axons derived from each eye invade target structures, and their endings are distributed in an overlapping manner; in the second phase, the terminal axons derived from the two eyes become segregated from each other in their target structures. Although injections of isotopes at closer intervals and shorter survival times are needed to define the duration of each phase, the available evidence suggests that the first phase may be relatively short, even in the protracted development of the rhesus monkey, whereas the second phase may last for several weeks. Thus, in the foetus injected at E110 and killed at E124, afferents from the LGd are distributed in an overlapping manner over the entire IVth layer of the visual cortex. Even 6 weeks later, on the seventh postnatal day, the segregation into monocular columns is far from

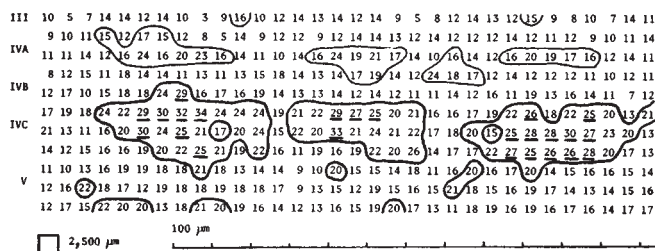


Fig. 3 Results of grain counts over a 0.8-mm² area of layers III, IVA, IVB, IVC and V of the primary visual cortex in monkey foetuses injected at E130 and killed at E144. The autoradiogram is photographed in dark-field illumination and its negative projected by a Prado (Leitz GMBH) apparatus on to grid paper in which each square corresponds to 2,500 µm² area of the cortex. Grains were counted in 352 unit areas along a 1.6-mm length of the calcarine cortex. Areas with 15 grains or more per 2,500 µm² in layer IVA are outlined by a thin line. Areas with 20 grains or more per 2,500 µm² in layer IVC are outlined by a thick line. Areas with 25 grains or more are underscored by a short, thick line. The distance between the two regions of highest grain counts that correspond to the pair of ocular dominance columns is approximately 500 µm. Note that the width of areas with higher grain counts is not half of the double column. Presumably, territories of terminals from the uninjected eye still considerably overlap the territories of injected eye and only a narrow, approximately 100–150-µm wide zone of highest and lowest grain counts may be considered to correspond predominantly to areas which receive projections derived predominantly from one eye.

complete¹². It is important to emphasise that although differentiation of pathways begins later in the cortex than in subcortical structures, the initial segregation of binocular connections in the cortex nevertheless begins well before birth—that is, before visual experience.

Thus the effect of monocular deprivation on the relative size of territories corresponding to each eye¹² may not necessarily involve the induced expansion of territories related to the functional eye. Rather, since domains of ocular representation overlap with each other during ontogeny (Fig. 3), it is possible that the territory of the functional eye remains arrested at a given developmental

stage, while the territories of the deprived eye shrink. In this context, our developmental data support the hypothesis that alterations in the relative width of ocular dominance columns could be degradative rather than inductive.

Recently, autoradiographic studies have demonstrated that radial columnar organisation is not confined to the primary visual cortex but is a generalised characteristic of the mammalian neocortex^{15,16}. For example, in the motor and prefrontal cortex of the neonatal monkey, callosal and cortico-cortical connections form well developed, sharply delineated columns whose development at this age is much more advanced than that of the ocular dominance columns of the visual cortex¹⁶. The precocious development of columns in the frontal lobe of the monkey adds further support to the notion that columnar organisation of the cortex may be largely specified innately. The significant alteration of monocular dominance columns by unbalanced visual stimulation during early stages of postnatal life in primates may occur because columns are not yet fully developed.

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Mayan chronology and 'the spectrum of time'

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By applying methods used in varve¹ and tree-ring² analysis to link the floating Mayan chronology with the anchored chronology of calculated planetary positions a new correlation has been derived. The intervals between certain Mayan dates are known to be multiples of the synodic periods of several planets. Mayan astronomers are assumed to have observed close conjunctions of two or more planets; this is tested by comparisons with observed or calculated conjunctions. I conclude that the Mayan dates as conventionally expressed are 27.3 yr too old. The suggested correlation number is 594,250 ± 1 d, the difference between the Julian and the Mayan day numbers.

DATES inscribed on numerous Mayan monuments in Central America during the first millenium AD can readily be converted to days in a floating time scale, but the correct dates in the Christian calendar have hitherto been uncertain. Many of the dates are now known to refer to the lives of the local rulers, but a few are 'astronomical'. Those dates of astronomical significance have been investigated in a computer study by Kelley and Kerr³, who, by knowing the mean planetary periods, have determined which planets are associated with certain of the dates.

Any Mayan date, such as 9.16.4.10.8. (the base of the Dresden Codex 'Eclipse' table), can thus be converted to the number of days elapsed since the starting point of Mayan chronology: this date, 9.16.4.10.8., is thus equivalent to a Mayan day number (MDN) of 1,412,848. Christian dates can similarly be converted to Julian day numbers (JDN), and to solve the Mayan Correlation Problem we must

determine for the Classic period the correct constant difference between any linked pair of these day numbers.

Other chronologies

The conventional equation is that of Goodman–Martinez–Thompson which makes the date 9.10.0.0.0. (MDN 1,368,000) equivalent to January 22, AD 633 (JDN 1,952,283); the Thompson correlation is thus 584,283 and this is usually regarded as correct by the sixteenth century, when Spanish interpreters attempted to explain the Aztec calendar.

Astronomers have not been satisfied with this equation and each specialist now seems to have a separate solution. In the recent AAS symposium⁴ at Mexico at least six were discussed, those of 487,410 (Owen), 482,699 (Smiley) and 584,283 (Hatch) being separately adopted in the published papers. The radiocarbon dates, combined with archaeological opinion, suggest that the correlation of 584,283 is not more than 30,000 d in error, and the late Sir Eric Thompson admitted: "Any correlation, deduced on other grounds, that makes 9.10.0.0.0. coincide with a conjunction of Venus and Jupiter would have much to recommend it." (see ref. 3, p.197). These two limiting conditions suggested the present investigation.

Data for new correlation

Observations of planetary conjunctions from China and elsewhere have been received from Professor E. H. Schafer and other contributors to the 'Spectrum of Time' project⁵, and the positions checked with planetary positions calculated by Tuckerman⁶. All possible comparisons between the Mayan dates and the observations in the T'ang Histories were made by the writer for a range of sixty years either side of the conventional date, and one solution only (594,250 ± 35) provided a consistent correlation for several conjunctions. The Venus–Jupiter conjunction of 650 was one recorded by the Chinese; this conjunction was later regarded by Masha'allah⁷ as signifying the fall of the Sasanians and the rise of the Arabs. This correlation dates the Venus–Jupiter conjunction of AD 650 near the Mayan date (9.10.0.0.0.) required.

positions. Calculations show that in 660 Jupiter and Venus were in the same longitude at about June 3 and likewise Jupiter and Mercury during the last half of the month.

Evidence from Caracol

Table 1 lists the dates in the inscription of Caracol (stela 3) selected by Kelley and Kerr³ for their summary chart; also indicated are the names of planets selected by the computer analysis and assumed to be those observed and recorded on this ephemeris.

The calculated⁸ longitudinal conjunctions near the dates cited are shown for comparison. Planetary conjunctions are not necessarily single events, some recurring within a few months or years so that discrepancies of up to 30 d are reasonable, but Mayan dates seem to have been adjusted slightly for some unknown reason. The stela can be interpreted in the light of the correlation⁸. In 653 Kelley's chart includes the date in his Saturn but not his Mercury column; although Jupiter was in conjunction with Mercury but not Saturn, Kelley's expectations in including the date in both his Saturn and Venus tables are confirmed by a Saturn–Venus conjunction on October 4 at a longitude separated by 45° from the other conjunction.

The Dresden Codex

The two dates in the Dresden Codex when most planets (according to the calculated periods of ref. 3) were involved can now be aligned on the new time scale as follows:

$$3.16.14.11.5. = 1,272,465 \text{ (MDN)} = 1,866,715 \text{ (JDN)} \\ = \text{October 15, 398}$$

$$9.12.10.16.9. = 1,386,329 \text{ (MDN)} = 1,980,579 \text{ (JDN)} \\ = \text{July 13, 710}$$

The former date is included in every planetary column except that for Jupiter (ref. 3, p. 210–211), and indeed it is found from Tuckerman that in November and December in that year all planets except Jupiter were in the same longitudes. A similar situation occurred in late June 710, Jupiter this time being involved as well; this is no doubt why calculations were made by I-Hsing at about AD 710, according to Needham⁹, concerning the date of the last

Table 1 Some dates from the inscription of stela 3 at Caracol

Mayan date	Planets involved	MDN (+594,250)	Date	Approximate dates of conjunction (after Tuckerman ⁶)
9.6.12.4.16.	Saturn–Venus	1,937,866	August 3, 593	July, 593 Saturn–Venus–Mercury
9.7.14.10.8.	Jupiter–Venus	1,945,898	July 31, 615	August 27, 615 Jupiter–Venus
9.9.10.0.0.	Saturn–Jupiter–Mercury	1,958,650	June 29, 650	June 9, 650 Saturn–Jupiter–Mercury
9.9.13.4.4.	Saturn–Jupiter–Venus	1,959,814	September 4, 653	June 12, 650 Saturn–Jupiter
9.9.14.3.5.	Saturn–Mercury	1,960,155	August 12, 654	Early September, 653 Mercury–Jupiter
9.9.18.16.3.	Jupiter–Venus	1,961,853	April 6, 659	August 16, 653 Saturn–Sun
9.10.0.0.0.	Saturn–Jupiter–Venus	1,962,250	May 9, 660	October 4, 653 Saturn–Venus
				July 30, 654 Saturn–Mercury
				March 13, 659 Jupiter–Venus
				June 3, 660 Jupiter–Venus
				October 24, 660 Saturn–Venus

Another conjunction now brought into alignment with a Mayan date in this way is that of 660, one not specifically recorded in the Old World. This conjunction, with a similar one two years later, appears to explain a letter of the Syrian Bishop Sebkht written about 662. Sebkht had been asked by the priest Basil from Cyprus whether there was any possibility of a conjunction of all the planets. John Philoponus in his polemic against Proclus had earlier mentioned a conjunction of 7 planets (that is including the Sun and Moon) in Taurus "in our time in the year 245 of Diocletian" (AD 529) and Sebkht in his reply confirmed this conjunction using contemporary tables of planetary

general conjunction. By multiplying the planetary periods together I-Hsing made it 96,961,740 yr ago, a Chinese calculation nearly as extravagant as corresponding calculations by the Mayans. There was also a visible eclipse of the Sun in 710 on May 3 and this is possibly referred to in the still undeciphered text.

Many other conjunctions can be dated in the monuments. In the Dresden Codex the earliest is that of June 8, 293 which is the exact date of a Jupiter–Mercury conjunction; the conjunctions of this year were observed by the Chinese¹⁰ who reported merely that "Saturn, Jupiter and Venus assembled at the 19th and 18th lunar mansions".

The latest Long Count date in this Codex is that of AD 1238 according to the 594,250 correlation suggested here. This is the date (9.16.4.10.8.) already mentioned as the base of the Eclipse table (ref. 3, p. 189). This does not coincide with an eclipse in the Goodman—Martinez—Thompson correlation, but on the new correlation there was a lunar eclipse visible to the Mayans before the following sunrise (January 2). The Dresden dates will be discussed separately, but, on this new basis, the Eclipse Table would have provided the Maya with predictions of every important lunar eclipse visible to them (± 2 d) for over 30 yr; the table also predicted almost every eclipse visible in other parts of the world, and it seems probable that the illustrations represented an unsuccessful attempt to differentiate between the two classes. The new correlation likewise makes possible a new interpretation of other dates associated with glyphs of the Sun, the Moon, Venus and eclipses.

Conclusion

Cross correlation of calculated conjunctions with the observed Mayan dates confirmed that there was no alternative solution within 250 yr using the known cycles of 60 and 24 yr in Saturn—Jupiter and Venus—Jupiter conjunctions.

The Goodman—Martinez—Thompson solution therefore needs an addition of 27.3 yr, corresponding to my suggested correlation of 594,250 d.

The various dates examined by Kelley, and other dates in the Dresden Codex and elsewhere, have been tabulated¹¹, and longitude—time charts have been drawn for the planets, the Sun and the Moon. Computer comparison with the associated glyphs should thus make it possible to identify the glyphs for the remaining planets, and, using the known dates of comets¹² in the Old World, to identify the glyph for comet. The writer would be very grateful for any collaboration in this project.

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Geochemistry and diagenesis of deep-sea sediments from leg 35 of the Deep Sea Drilling Project

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The principal cause of ^{18}O depletion, Ca^{2+} increases, and Mg^{2+} decreases in the pore fluids of cores from leg 35 of the Deep Sea Drilling Project (DSDP) is the alteration of basalt, volcanic ash, and igneous components of terrigenous material. Post-depositional diagenetic reactions have produced smectites, zeolites, K-feldspar, and celadonite as the main authigenic products. $^{18}\text{O}/^{16}\text{O}$ data from calcite and clay minerals suggest that these reactions took place over a range of times, temperatures (10–60 °C), and pore fluid $^{18}\text{O}/^{16}\text{O}$ compositions. At site 323, the sediments 30–74 m above basement are enriched in Mn, Fe, Cu, Ni, Co, Mo and Sr. Below these horizons, Ba shows a sharp increase towards basement. The $^{13}\text{C}/^{12}\text{C}$ values ($\delta = -18.4$ to -21.2‰ PDB) of authigenic calcites in the sediments reflect an organic matter source for the carbon.

LAYER IIA (extrusive volcanics) and layer I (sediments) of the oceanic crust undergo major chemical and mineralogical transformations from the time of their extrusion or deposition to the time of their subduction. These transformations cause chemical changes in the pore fluids which, in turn, produce chemical changes in the oceans. The directions of chemical exchanges are known, but the locations and the sizes of the major chemical sinks and sources remain poorly defined^{1–3}.

Diagenetic reactions are recorded by chemical, mineralogical, and isotopic changes in the sediments, in their pore fluids, and in the underlying basalts. Decreases in dissolved sulphate concentration, along with increases in dissolved ammonia and alkalinity, are principally a reflection of sulphate reduction by bacteria^{4,5}. Increases in calcium and decreases in magnesium, potassium, and $^{18}\text{O}/^{16}\text{O}$, however, are caused by mineralogical transformations in the sediments and basalts. Increases of calcium with depth are not always caused by dissolution and/or recrystallisation of CaCO_3 (refs 5 and 6) but can originate from

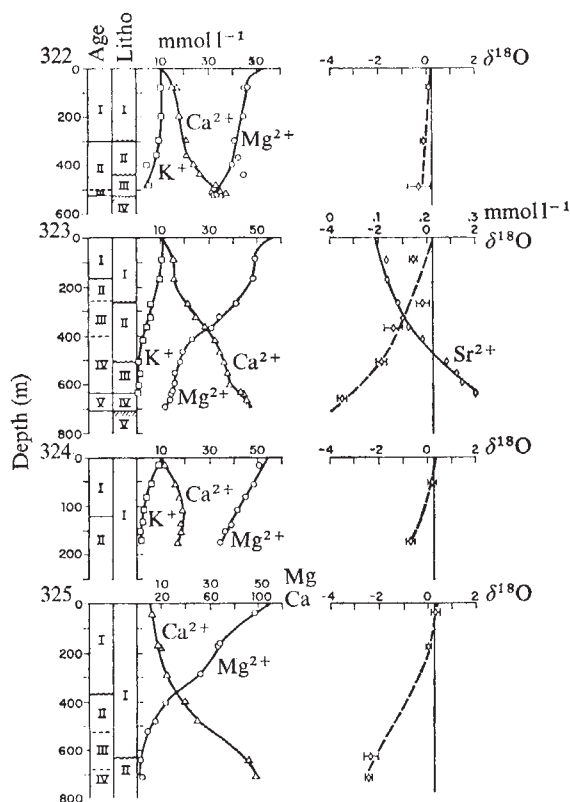


Fig. 1 Ca^{2+} , Mg^{2+} , K^{+} and $\delta^{18}\text{O}$ gradients in interstitial fluids of leg 35, sites 322, 323, 324 and 325 DSDP.

the alteration of calcium-containing igneous phases. With the exception of sediments overlying evaporites, and some rapidly depositing sediments, a decrease in magnesium almost always accompanies an increase in calcium. Silicate transformations that involve magnesium exchange for calcium, as well as potassium and sodium uptake, are the most probable cause for the observed pore water gradients.

It was long thought that the amount of solids undergoing alteration should be small and, therefore, difficult to detect. With the discovery of $^{18}\text{O}/^{16}\text{O}$ decreases in the pore fluids that accompany major cation gradients, however, it became apparent that in many instances the amount of solids involved in the reactions had to be large⁷, and it has been shown that the observed $^{18}\text{O}/^{16}\text{O}$ and major cation gradients are caused mainly by the alteration of volcanic ash or basalt⁸⁻¹⁰.

Here we present the highlights of an intensive collaborative effort to delineate more clearly the major sinks and sources for the elements mentioned above. The focus is on the elemental and stable isotope chemistry of the interstitial waters and the chemistry and mineralogy of the sediments and basalts, leg 35 of DSDP in the Bellingshausen abyssal plain area of the southern Pacific Ocean.

Details of the sites

The changes in Ca^{2+} , Mg^{2+} , K^{+} and $\delta^{18}\text{O}$ of the pore fluids are shown in Fig. 1, and the age and lithology of leg 35 sites in Table 1. All four sites show increases in Ca^{2+} and decreases in Mg^{2+} , K^{+} , and $\delta^{18}\text{O}$ with depth, but each site has its own signature. Clearly, the number and location of reaction zones are not the same in each site. Average slopes and changes in slopes of the concentration gradients are variable, being functions of extent and type of reactions, reaction rates and sedimentation rates, as well as the physical properties of the sediments. Dissolved Mg^{2+} reaches a concentration of almost zero only in site 325, which has the largest average sedimentation rate. A marked contrast in the amount of $\delta^{18}\text{O}$ depletion

exists between sites 322 and 323. Numerous and most extensive diagenetic changes were observed in site 323.

Site 323

Studies of the diffusional transport properties of the sediments¹¹ have led to the conclusion that the dissolved strontium gradient (Fig. 1) > 639 m arises essentially from diffusion from a 'point' source, probably located in the Sr-enriched sediments just below this boundary (Fig. 2). By comparing the Ca^{2+} , Mg^{2+} , and K^{+} gradients in the pore fluids with the Sr^{2+} concentration depth profile, changes in slope can be interpreted with confidence in terms of reaction zones. Accordingly, three principal zones can be recognised:

a, the rapidly deposited Pliocene section (75–100 m); *b*, the zone of silification (400–510 m); and *c*, the basal sediments, consisting mostly of authigenic smectite and some clinoptilolite (640–700 m) and/or the underlying partially weathered basalts.

In zone *a*, weathering of plagioclase and/or mafic minerals is probably the source for Ca^{2+} , and authigenic clays are the most likely sinks for Mg^{2+} and K^{+} . In zone *b*, at ~ 450 m, Kastner¹² demonstrated from SEM photographs and X-ray energy dispersive chemical measurements that weathering of plagioclase and dissolution of nannoplankton are the sources of Ca^{2+} and authigenic smectite and K-feldspar are the sinks for Mg^{2+} and K^{+} .

An abrupt change in mineralogy of the sediments takes place at 639 m in zone (*c*), immediately below a large sedimentation hiatus of ~ 35×10^6 yr (ref. 13). Above, in the clay-size fraction, illite and mixed layered illite-smectite of 60–70% expandable layers are the most abundant clay minerals. This is also reflected in the relatively high K/Al ratios in the clay fraction (Fig. 2). Below, the dominant clay mineral is smectite and the K/Al values are low in the clay-size fractions. Of interest, however, is the observation that the K/Al ratio of the bulk sediment is nearly the same above and below this boundary¹⁴. Evidently the lower sediment has its K_2O preferentially located in relatively coarse phases (K-feldspar and clinoptilolite)¹³.

Interpretation

The above mineralogical and chemical differences leave little doubt about the diagenetic nature of the sediments below

Table 1 Details of the sites

Site 322—60°01.45'S, 79°25.49'W; 5,026 m	
Age	I: Pleistocene/Pliocene II: Miocene III: Oligocene?
Lithology	I: sand; silt; clay II: claystone III: sandstone and claystone IV: basalt
Site 323—63°40.84'S, 97°59.69'W; 4,993 m	
Age	I: Pleistocene/Pliocene II: Late Miocene III: Middle Miocene IV: Early Miocene V: Palaeocene and older
Lithology	I: clay; silt; sand II: diatomite; claystone; chert < 400 m III: claystone IV: zeolitic clay V: basalt
Site 324—69°03.21'S, 98°47.20'W; 4,449 m	
Age	I: Pleistocene II: Pliocene
Lithology	I: clay; silt; claystone
Site 325—65°02.79'S, 73°40.40'W; 3,745 m	
Age	I: Pleistocene/Pliocene II: Late Miocene III: Middle Miocene IV: Early Miocene
Lithology	I: clay; silt; claystone II: claystone and silicified claystones

the hiatus. Changes in the mineralogy of the clays suggest a volcanic source¹³. Glass and palagonite have been reported in these sediments¹². Donnelly and Wallace¹⁴, however, emphasise the essentially isochemical nature of the bulk sediments above and below the hiatus, particularly with regard to the Ti/Al ratio (0.031) and the contents of K_2O , Na_2O , and MgO . Exceptions, of course, are the contrasting values of Fe, Mn, and P. On the basis of these data, they suggest that the sediments < 639 m are mainly of a terrigenous origin but have undergone substantial alteration to form the above-mentioned suite of minerals.

Pore water concentration gradients in Ca^{2+} , Mg^{2+} and K^+ in zone c cannot be interpreted with certainty as involving reactions in these sediments but may be related to ongoing alteration of underlying basalts, which show the presence of alteration products^{12,13}. Typically, $\delta^{13}C$ values in calcite in the uppermost basalts are as low as -3‰ (PDB)¹⁵, probably implying biogenic degradation of organic matter. The pyroxenes of the basalts are more extensively weathered than the plagioclases. Both are likely to provide Ca^{2+} to the pore fluids. Smectites in basalt veins may constitute important sinks for Mg^{2+} . The K^+ depletion observed < 639 m may be related to uptake in authigenic zeolites in the basal sediments and/or authigenic K-feldspars.

The large sedimentation hiatus previously mentioned implies either a long period of non-deposition or rather slow sedimentation rates and subsequent erosion by bottom currents during the onset of the circumpolar current. In either case, part or all of the alteration observed below the hiatus or in the basal sediments may have occurred before the Miocene, that is, before the renewed buildup of sediments. Observed pore water gradients < 500 m may then be caused principally by continued alteration of the underlying basalts.

Between 639 and 670 m, 30–74 m above the basement, the solids showed an enrichment in transition elements and strontium^{13,14,18}. Below this, barium shows a sharp increase towards basement (Fig. 2). We interpret this in terms of hydrothermal events in the underlying basalts which have led to an upward advection of these hydrothermal fluids with the subsequent precipitation of barite near the basalt-sediment interface and the formation of transition metal oxides in the oxygenated upper sediments. The observations of the occurrence of celadonite¹² and saponite¹³ in the basalts, and of well crystallised clay minerals in the basal sediments¹⁹ and basalts¹² are consistent with this interpretation.

Oxygen isotope data

The oxygen isotope data of the pore waters (Fig. 1) indicate a gradual decrease with depth. Alteration of continental detritus > 639 m does not seem to have contributed greatly to the $\delta^{18}O$ depletion. This is particularly evident from oxygen isotope data on the 0.3–0.7- μm size fraction of quartz, which do not show any increase in $\delta^{18}O$ with depth¹⁶. For the 60 m of authigenic basal sediments, the oxygen isotope data favour an ash precursor over a terrigenous one. Volcanic ash has a lower $\delta^{18}O$ ($\sim +10\text{‰}$) than terrigenous material ($\sim +15\text{‰}$). Thus, alteration of a given amount of ash to smectites with a $\delta^{18}O = +18.7\text{--}25.1\text{‰}$ (ref. 15) would lower the $\delta^{18}O$ of the pore waters more than the alteration of the same amount of terrigenous material. The major observed alteration, however, probably occurred before the resumption of sedimentation in the Miocene, as has been suggested before. During the long hiatus and the period of slow accumulation of the basal sediments, all or most of the ^{18}O depletion of the pore fluids must have been obliterated by diffusional exchange with the overlying ocean. Therefore, the observed ^{18}O depletions must have occurred after the resumption of sedimentation.

The oxygen isotope depletions demand alteration of solid phases on a large scale⁸. We calculate alteration of a minimum of 20 m of basalt, 35 m of ash, or 80 m of terrigenous material at site 323 (ref. 20). Note that the order of effectiveness in pro-

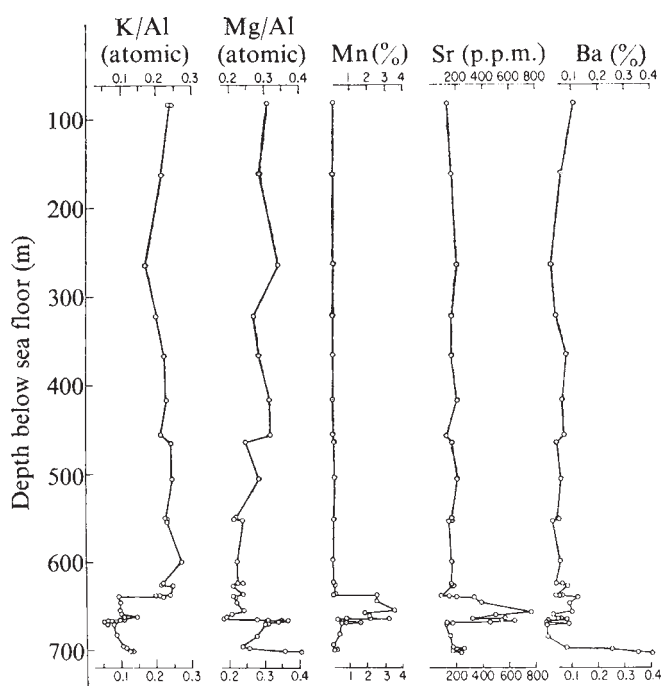


Fig. 2 Chemical composition of clay-size fraction, site 323.

ducing ^{18}O depletions in the pore fluids is: basalt > ash > terrigenous material⁸.

From the above considerations, we conclude that the alteration reactions in the sediments cannot account for all the $\delta^{18}O$ depletion of the pore waters. The underlying basalts, however, show the presence of several authigenic minerals. Of the 28 m of basalt cored, only 10 m were recovered. Vennum²¹ suggested, especially on the basis of increased drilling rates, that one or two sills may have been drilled without actually reaching oceanic basement. Increased drilling rates suggested the presence of interlayered sediments and/or highly altered basalts. We propose that much of the observed $\delta^{18}O$ depletion must have its origin in the continuing alteration in the underlying basalts.

Smectite from the basal sediments ($\delta^{18}O = +18.7\text{--}25.1\text{‰}$), as well as celadonite ($\delta^{18}O = +19.6\text{‰}$), saponite ($\delta^{18}O = +24.2\text{‰}$), and calcite ($\delta^{18}O = +26.5\text{--}31.4\text{‰}$) from the basalts formed at relatively low temperatures. If alteration to these products occurred in an infinite reservoir of deep ocean water with a $\delta^{18}O = 0\text{‰}$, the temperature range must have been 10–60 °C. Because the observed $\delta^{18}O$ values of the pore fluids are as low as -3.5‰ (Fig. 1), it is apparent that an infinite reservoir of water was probably not involved. Consequently, the alteration products may have formed over a smaller temperature range than that estimated above. The range in $\delta^{18}O$ of the calcite veins and clay minerals requires formation at different temperatures or in the presence of pore fluids with different $\delta^{18}O$ values and, therefore, at different times in the history of the site. In the basalts, an earlier formed vein ($\delta^{18}O = +31.4\text{‰}$) is cut by a later vein ($\delta^{18}O = +29.5\text{‰}$)¹⁵. It appears that the earlier vein may have formed in contact with open ocean water at 10 °C and the later vein at a higher temperature ($\leq 20\text{ °C}$) in the presence of pore fluid with a $\delta^{18}O$ of 0‰ or lower. Such a sequence of events probably applies to the several generations of clay formation in the basalts and sediments. The hydrothermal event that caused the enrichment of transition metals and strontium 30–74 m above basement may also have been responsible for the formation of the celadonite and saponite with moderately low $\delta^{18}O$ values¹². Based on the above oxygen

isotope values, the temperature of the hydrothermal fluids could not have been $> 60^{\circ}\text{C}$.

Other sites

Based on chemical gradients in the pore fluids (Fig. 1), the main reaction zone in site 322 is in the lower part of the sediment section or in the underlying basalt. The $\delta^{18}\text{O}$ depletion in site 322 is very small, so that relatively little alteration appears to have occurred. Data on dissolved calcium and $\delta^{18}\text{O}$ in site 325 suggest the presence of reactive sediments below the section cored.

The significant gradients observed in the pore fluids of site 324 probably arise not only from volcanic glass alteration that has been observed in the recovered sediments of this site but also from reactions in deeper, unrecovered portions.

$\delta^{13}\text{C}$ values of -18.4 to -21.2% (PDB) of authigenic calcite in the sediments of sites 323 and 325 indicate that organic matter was the major source of carbon for these calcites¹⁵.

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letters to nature

Transient radio source near the galactic centre

A VARIETY of interesting objects have been discovered in recent years within several arc min of the galactic centre. These include the main (east and west)¹ components of the radio source Sgr A, a compact radio source² at the centre of Sgr A West which may be the galactic nucleus, and a group of infrared sources³ in a region of $\sim 20''$ diameter surrounding this compact radio source. In February 1975 Eyles et al.⁴ discovered the transient X-ray source A1742–28 near the galactic nucleus. We report here the detection of a radio transient source which we believe is very likely to be associated with this X-ray source. Although we have evidence that this transient radio source lies close to the centre of the Galaxy, it is clearly not to be identified with the nucleus itself.

Observations of the galactic centre region have been made at intervals since June 1, 1974 using the Jodrell Bank radio-linked interferometers in a programme of investigations of the

compact nuclear source. The Jodrell Bank (MK IA or MK II)-to-MK III interferometer (24-km baseline) was operated at 0.96 and 1.66 GHz while the MK IA-to-Defford interferometer (127-km baseline) was operated at 0.408 GHz. A summary of the dates of observation and of the parameters of the baselines used is given in Table 1. The observation on March 30, 1975 at 0.96 GHz revealed the emergence of a new radio source near the already known compact nuclear source. The signature of a relatively weak double source was clearly seen in the amplitude and phase data on this day; the amplitude data are reproduced in Fig. 1 along with the double source model which is the best fit to the observations. All the other observations of this region at the various times listed in Table 1 did not detect a signal at the position of the transient source and only provided upper limits to the emission at these times.

The amplitude and phase data for March 30, 1975 were used to determine an accurate offset of the transient source from the compact nuclear source whose position is given as RA = 17 h 42 min 29.291 $\pm$ 0.005 s, dec. (1950) = $-28^{\circ}59'$

Table 1 Radio observations of the transient source A1742–28

Date	Frequency (GHz)	Observed flux density (mJy*)	Interferometer	Projected baseline	
				shortest ($\lambda \times 10^3$)	longest ($\lambda \times 10^3$)
June 01.0, 1974	1.66	< 55	MK I–MK III	60	132
November 30.6, 1974	0.408	< 50	MK I–Defford	27	86
March 30.2, 1975	0.96	220 (480†)	MK II–MK III	34	75
May 3.1, 1975	0.408	< 50	MK I–Defford	27	86
June 20.9, 1975	0.96	< 26	MK II–MK III	34	75
July 6.8, 1975	0.96	< 38	MK II–MK III	34	75
September 10.7, 1975	0.96	< 30	MK II–MK III	34	75

*1 mJy = $10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$.

†Flux corrected for resolution of the interferometer.

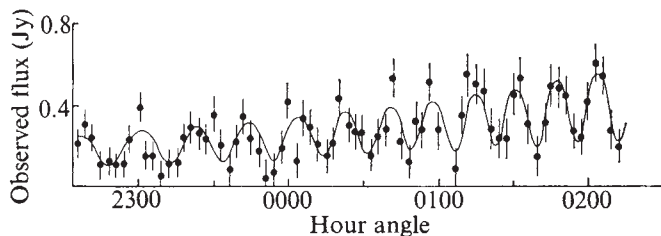
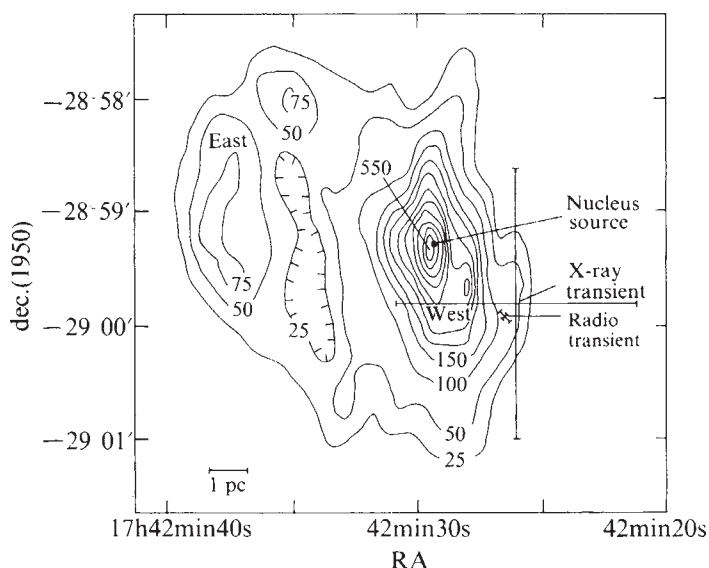


Fig. 1 The amplitude and phase plots obtained at 0.96 GHz with the MK II-MK III interferometer (24-km baseline) on March 30, 1975. The interferometer was set on the position of the compact source at the galactic nucleus (RA = 17 h 42 min 29.29 s, dec. (1950) = $-28^{\circ}59'17.6''$). The beat pattern in the interferometer output results from the additional contribution of the new transient source. A model of a double source with a separation $53''$ at p.a. = 223° is shown as a full line in the amplitude plot; the intensities of the nuclear and transient source are 0.18 and 0.39 Jy respectively; both have a Gaussian shape with a diameter between half intensity points of $1.5''$.

$17.6 \pm 0.1''$ by Balick and Brown². We have measured the offset of the transient source as $53 \pm 3''$ in position angle (pa) = $223 \pm 2^{\circ}$ which corresponds to $\Delta RA = -2.77 \pm 0.22$ s and $\Delta dec. = -37.4 \pm 2.8''$. This places it at RA = 17 h 42 min 26.52 $\pm$ 0.22 s, dec. (1950) = $-28^{\circ}59'55.0 \pm 2.8''$ which lies within the larger errors of the position of the X-ray transient at RA = 17 h 42 min 26.0 $\pm$ 4.8 s, dec. (1950) = $-28^{\circ}59'48'' \pm 1'12''$ as shown in Fig. 2. The X-ray position uncertainty also includes the compact nuclear radio source, but it will be argued below that the radio transient source is associated with the X-ray transient.

The run of radio and X-ray intensities of the transient source are compared in Fig. 3. At radio wavelengths an upper limit is plotted for each date except the detection on March 30, 1975. At X-ray wavelengths⁵ the emission decayed rapidly for 40 d with an e-folding time of 12 d followed by a slower decay for 140 d with an e-folding time of 90 d. It is of course not known where, between February 22 and March 30, the maximum in the radio emission occurred. Figure 3 shows, however, that the radio decay does not mirror the X-ray decay; between March 30 and May-July the X-ray intensity falls only by a factor of ~ 2 while the radio has fallen by a factor of ~ 6 . Additional upper limits to the X-ray emission outside the

Fig. 2 A 5.0-GHz map of the Sgr A area by Ekers *et al.* showing the position of the nuclear source (filled circle) and the new transient source (cross); the size of the cross indicates the position uncertainty of the transient source. The position and position error of the X-ray transient source A1742-28 as given by Eyles *et al.* are indicated as a large cross.



time of the main transient activity have been supplied by Dr P. W. Sanford of the Mullard Space Science Laboratory and Dr M. J. L. Turner of Leicester University.

There are two arguments which suggest an association between the radio and X-ray transients. The first is that, in spite of the sporadic nature of the radio and X-ray observations, there is general agreement in the period of the activity at both radio and X-ray wavelengths; at times beyond ± 40 d of the X-ray eruption there was no detectable radio and only weak X-ray emission (Fig. 3). The second argument is the close agreement to better than $7''$ in each coordinate between the

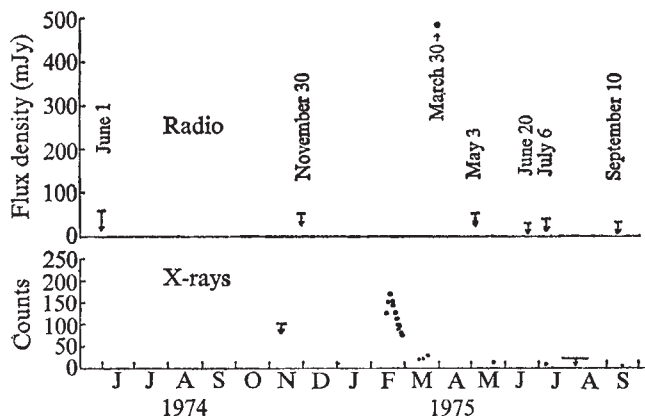


Fig. 3 A plot of the observations of the X-ray and radio intensity of A1742-28 as a function of time. The frequencies of the radio observations are given in Table 1.

measured radio and X-ray position of the transient objects. This implies that liberal systematic errors were assigned by Eyles *et al.*; the true error may have been composed only of their random error of $10''$.

The interferometric data show that the angular diameter of both the transient source and the nuclear source are $1.5 \pm 0.3''$ at 0.96 GHz. The true flux of the source on March 30, 1975 was 0.48 ± 0.07 Jy when corrected for the resolution of the interferometer. Available information on the diameter of the nuclear source as a function of wavelength, λ , indicates that its diameter increases as λ^2 ; this is the dependence expected for interstellar scattering. The high electron line-integral between the Sun and the galactic centre can readily account for the observed scattering. The close similarity in the angular sizes of the transient and nuclear sources which lie so close together in the sky would argue that their diameters have been affected by the same clouds of interstellar electrons presumably lying near the galactic nucleus. We therefore infer that the transient source is also at the galactic centre. It is of interest to note that the radio outburst of Cyg X-3 was also broadened (to a half power width of $2''$) by interstellar scattering at 0.408 GHz (ref. 6). Cyg X-3 is at a distance of ~ 10 kpc also and lies behind the Cyg X radio H II region; its intrinsic diameter is $\lesssim 0.1''$ (refs 6 and 7).

The present observations provide strong evidence that the radio and X-ray sources are related and that they lie $53 \pm 3''$ from the true galactic nucleus as defined by the compact radio source. Although the transient source lies within the outer contours of the Sgr A West radio source, there seems to be no structural feature in the 5.0 GHz map of Ekers *et al.*¹ (Fig. 2) which can be identified with it; nor has any intense infrared source been reported⁸ at this position although it lies on the infrared ridge at $2.2 \mu\text{m}$. If it is at a distance from the centre of 2.5 pc which corresponds to its projected angular distance then it is within a region of high stellar density of $10^5 M_{\odot} \text{pc}^{-3}$ in the model of Oort⁹; this density is a factor of 10^8 greater than in the solar vicinity. One of the stars in this nuclear concentration is presumably responsible for the radio and X-ray outburst. Braduadi *et al.*⁵ suggest that it may be a

binary system containing a compact object with variable mass transfer because of its similarity with 3U1543-47.

The X-ray and radio properties of this transient object do not seem to be unique even though it is occurring in what might be considered a unique part of the Galaxy. Its intrinsic radio luminosity ($5 \times 10^{33} \text{ erg}^{-1}$ between 1 and 100 GHz if assumed to have a flat spectrum) lies between that of bursts observed in Cyg X-1 and Cyg X-3. The apparent association in time between outbursts or changes in intensity at radio and X-ray wavelengths observed here is also found in Cyg X-1 and the new transient source A0620-00 (refs 10, 11). The intrinsic peak X-ray luminosity of $3 \times 10^{38} \text{ erg}^{-1}$, however, is at the top end of the range measured for known X-ray sources^{4,5}, if it is placed at the galactic centre, as the present results indicate.

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Steepening of the γ -ray background spectrum from local γ -ray production

BECAUSE the Solar System lies in a spiral arm of our Galaxy, much of the γ -ray flux arriving at large angles to the galactic plane is of local origin. We have tried to calculate this contribution to the flux, and find that the effect is particularly pronounced for γ rays of energy $> 100 \text{ MeV}$. This implies a steeper spectrum for extragalactic γ rays than has been hitherto expected.

Beside γ radiation originating from the Galaxy there is evidence for a general γ background. The differential energy spectrum of this diffuse radiation can be represented by a power law:

$$dJ/dE_\gamma = (2.7 \pm 0.5) \times 10^{-7} \times (E_\gamma/100)^{-2.4 \pm 0.2} \text{ photons cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ MeV}^{-1}$$

for photon energies between 35 and 170 MeV (ref. 1). Beside these results from the SAS-2 satellite experiment there are results from balloon experiments covering the range 1-100 MeV which show that this diffuse—for lower energies highly isotropic—radiation has an intensity above the line extrapolated from high energy X-ray data. The most prominent of the models proposed to explain this are (1) the decay of neutral pions produced by cosmic-ray interactions with the intergalactic gas at high redshifts (protar hypothesis)², (2) the decay of neutral pions arising from matter-antimatter annihilation³ and (3) pair production by cosmic-ray protons on the microwave

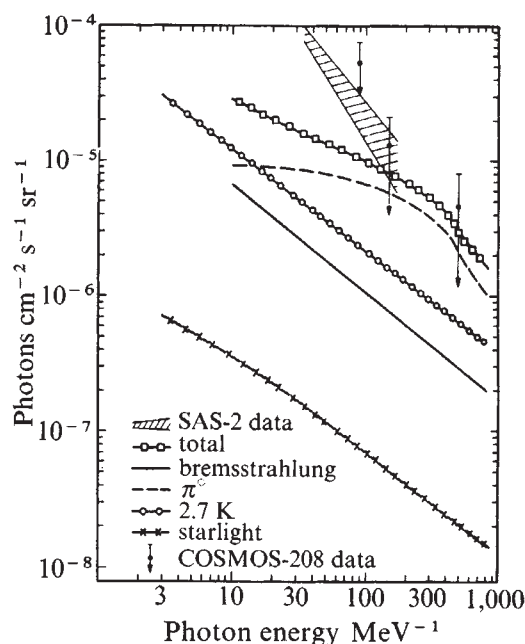
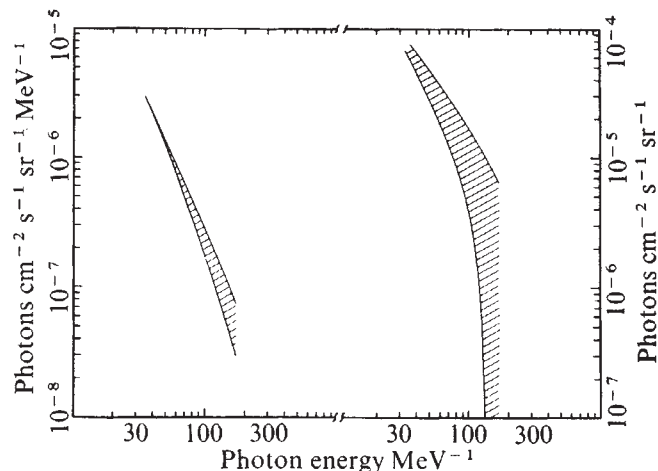


Fig. 1 Integral spectral energy spectrum of galactic γ rays from directions with high galactic latitude ($|b^{\text{II}}| > 30^\circ$) in comparison with SAS-2 and COSMOS-208 data. The half thickness of the relativistic electron disk has been taken as $z_B = 1 \text{ kpc}$.

background followed by an electromagnetic cascade involving the inverse Compton effect⁴. These all favour an extragalactic origin of the radiation.

Usually this diffuse background radiation is observed by pointing the detector axis away from the galactic plane in directions with high galactic latitude b^{II} . In the SAS-2 experiment all γ rays coming from regions with $|b^{\text{II}}| > 30^\circ$ have been attributed to the diffuse component. On the other hand, studies of starlight polarisation, Faraday rotation of pulsars and extragalactic radio sources⁵ and 21-cm line emission indicate that the Solar System is located inside a spiral arm of our Galaxy implying that even at high latitudes a part of the observed γ rays are produced in the local spiral arm. To estimate the contribution of these locally produced γ rays from high latitudes we calculate the differential and integral spectral flux from directions observed by SAS-2, namely I ($l^{\text{II}} = 0^\circ$, $b^{\text{II}} = 58^\circ$), II ($l^{\text{II}} = 285^\circ$, $b^{\text{II}} = 75^\circ$) and III ($l^{\text{II}} = 300^\circ$, $b^{\text{II}} = -45^\circ$), by taking into account bremsstrahlung⁶ and π^0 -decay⁷ in the

Fig. 2 Corrected ($z_B = 1 \text{ kpc}$) differential (left) and integral (right) energy spectrum of extragalactic γ rays.



interstellar gas and inverse Compton scattering of low energy starlight and black-body photons⁸ as well as the final angular resolution of the SAS-2 γ -ray detector which was treated as a two-dimensional Gauss function with a s.d. of 5°.

The astrophysical parameters contained in the emissivities of the various γ -ray production processes were calculated by using analytical formulae for the latitudinal distribution of stellar and interstellar matter^{9,10}. The most important derived parameters are the hydrogen column densities in the three directions I, II and III, which are 5.8×10^{20} , 5.0×10^{20} and 8.3×10^{20} atoms cm^{-2} respectively, the mean value of which, $N_H = 6.4 \times 10^{20}$ H atoms cm^{-2} , determines the absolute values of the π^0 -spectrum which, as can be seen from Fig. 1, is the main part of the total calculated spectrum for photon energies > 100 MeV. Checking these column densities with estimates from Lyman α absorption measurements^{11,12} ($N_H \approx 3 \times 10^{20}$ H atoms cm^{-2})—which provide only lower limits because of unseen gas beyond the observed stars—interstellar reddening data¹³ (N_H between 2×10^{20} and 9×10^{20} H atoms cm^{-2}) and 21-cm observations¹⁴ applying a factor 2 for the abundance of hydrogen molecules^{15–17} ($N_H \approx 8 \times 10^{20}$ H atoms cm^{-2}) we find reasonable agreement. The local energy spectrum of cosmic ray protons and α -particles is contained in the production rate of the π^0 -decay⁷; the interstellar electron spectrum has been taken as a power law with a spectral index 1.8 for energies < 2 GeV and 2.5 for energies > 2 GeV (refs 18 and 19).

It can be seen from Fig. 1 that for photon energies > 100 MeV the locally produced γ -ray component becomes dominant: at least we find that $51^{+21}_{-14}\%$ of the integral (> 100 MeV) spectral flux is of galactic origin where it should be noted that the error bars come from the uncertainties in the SAS-2 measurements. On the other hand at lower energies ($E_\gamma > 40$ MeV) only $21^{+4}_{-2}\%$ of the integral spectral flux is produced in the local environment of the Sun. If the upper limit at 500 MeV measured in the COSMOS-208 experiment²⁰ will be confirmed by future measurements we can conclude that at these energies the γ -ray flux from high latitudes arises predominantly from local events. Repeated calculations with different values of the half-thickness of the relativistic electron disk $z_B = 300$ pc and 500 pc lead to the same conclusion because only the black-body component—which has no significant influence on the upper part of the total spectrum—depends linearly from the values of z_B .

This effect of the increasing importance of locally produced γ -rays can also be seen by studying the ratio of the local and empirical differential spectral flux: at 40 MeV 6%, at 100 MeV $16^{+4}_{-2}\%$ and at 170 MeV $32^{+12}_{-6}\%$ are of galactic origin. In terms of the 'real' extragalactic γ radiation this means that the high energy end of the measured spectra must be corrected because of local events whereas at lower energies the measured spectra are not affected by galactic γ -ray production. This last result is in good agreement with isotropy measurements of this diffuse radiation showing that at low energies (< 10 MeV) $> 87\%$ of the measured flux is of extragalactic origin²¹.

In principle, by subtraction of calculated locally produced γ rays from the observed spectrum at ~ 100 MeV, one may obtain the 'real' extragalactic γ -ray spectrum, the discussion of which should make possible a distinction between the annihilation hypothesis and the protar hypothesis. The former gives rise to a steeper spectrum than predicted by the latter hypothesis³. We are quite aware of the fact that statistics of the SAS-2 as well as the COSMOS-208 data above 100 MeV are insufficient for conclusive arguments concerning these two alternatives. Nevertheless, to get a tentative result we have made this subtraction shown in Fig. 2 which seems to be more in favour of the annihilation hypothesis rather than the protar hypothesis. It will be most important to obtain further measurements to get better statistics on the energy spectrum > 100 MeV from high latitude directions as well as on the isotropy of arrival directions in this energy range.

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Particle creation and Dirac's large numbers hypothesis

IN connection with cosmologies based on the large numbers hypothesis¹ (LNH), Dirac² has suggested that continuous creation of matter is required. Two modes of particle creation have been proposed and cosmological models corresponding to each mode have been studied³. We demonstrate here that, within the context of the LNH, the number of particles in the Universe varies as expected ($N \propto t^2$) so that particle creation is unnecessary. Some unpleasant features of cosmologies based on the LNH are also discussed.

To appreciate fully the results to be presented for cosmologies based on the LNH, it will be valuable to review first the corresponding relationships in conventional cosmologies in which the gravitational constant, G , does not vary with epoch. For such models, the variation of the density with the age of the Universe is given by

$$G\rho t^2 = \text{constant} \quad (1)$$

This result is valid for early times (high densities); it is also valid now if the density is equal to the critical density ($\rho_c = 3H_0^2/(8\pi G)^{-1}$). Now, we will be interested in the variation with epoch of the number of particles in the Universe. By "in the Universe" we mean within the horizon or, up to a numerical factor, within the Hubble distance c/H . The volume of the Universe thus varies with epoch as $V \propto H^{-3} \propto t^3$ (see, for example, ref. 3). For a pressure-free (matter dominated (MD)) cosmological model, the particle density, n , is proportional to the density ρ . Then, from equation (1) we see that $n \propto \rho \propto t^{-2}$ so that the number of particles in the Universe increases as

$$N = nV \propto t^{-2}t^3 \propto t \quad (2)$$

The presence of the black-body radiation suggests that pressure played an important dynamical role in the early Universe. If the early evolution was radiation dominated (RD), then $n \propto \rho^{3/4} \propto t^{-3/2}$. In this case the number of particles in the Universe varies with epoch as

$$N = nV \propto t^{-3/2}t^3 \propto t^{3/2} \quad (3)$$

We will also be interested in the variation with epoch of the temperature, T , of the background radiation. Since the temperature varies with particle density as $T \propto n^{1/3}$, it follows that

$$\begin{aligned} \text{MD} : T &\propto n^{1/3} \propto \rho^{1/3} \propto t^{-2/3} \\ \text{RD} : T &\propto n^{1/3} \propto \rho^{1/4} \propto t^{-1/2} \end{aligned} \quad (4)$$

A reasonably accurate $t(T)$ relationship may be obtained if account is taken of the fact that the Universe is MD for $T \lesssim 10^4 T_0$ and RD for $T \gtrsim 10^4 T_0$ ($T_0 \simeq 2.7$ K).

$$\begin{aligned} \text{MD} : t &\simeq 10^{18} T^{-3/2} \text{ s} \\ \text{RD} : t &\simeq 10^{20} T^{-2} \text{ s} \end{aligned} \quad (5)$$

Recall for future reference that primordial nucleosynthesis occurs when $10^8 \lesssim T/T_0 \lesssim 10^9$ at which time the age of the Universe is $10 \lesssim t(\text{s}) \lesssim 10^3$ (see, for example, ref. 4).

We now turn to a re-examination of these results in a cosmology based on Dirac's large numbers hypothesis¹. Dirac noticed that the ratio of the present age of the Universe (t_0) to an atomic unit of time ($t_e \equiv e^2/m_e c^3 \simeq 10^{-23}$ s) is a large dimensionless number (LDN).

$$\tau_0 \equiv t_0/t_e \simeq 10^{40} \quad (6)$$

In the past (future) this ratio had (will have) a different value; $\tau \equiv t/t_e$ varies with epoch. Dirac was led to suggest that all such LDN vary with epoch; if the present value of a LDN is $N_0 \simeq \tau_0^a$ then, $N \propto \tau^a \propto t^a$. One of the most striking consequences of this assumption is that the gravitational 'constant', G , must vary with time^{1,2}: $G \propto t^{-1}$. An immediate consequence for cosmologies based on the LNH is that: $\rho \propto t^{-1}$. This rapid variation of epoch with density ($t \propto \rho^{-1}$ compared with $t \propto \rho^{-1/2}$ previously) has far-reaching effects to which we now turn.

The number of observable particles in the Universe is an example of a LDN. At present, $N_0 \simeq \tau_0^2$, so that the LNH requires that $N \propto t^2$. This is a more rapid variation of N with t than given by equation (2) and Dirac² was led to conclude that particle creation was necessary. (It should be noted that Jordan³ also proposed a cosmology based on the LNH. He noted that $G \propto t^{-1}$ in such cosmologies and for the reasons just outlined concluded that particle creation was required.) The result contained in equation (2) depends crucially, however, on the assumption of a constant G . If $G \propto t^{-1}$ we obtain for a MD cosmology

$$N = nV \propto \rho V \propto t^{-1} t^3 \propto t^2 \quad (7)$$

This result is in perfect agreement with the LNH; particle creation is unnecessary. Note that for an RD model a somewhat different result is obtained

$$N = nV \propto \rho^{3/4} V \propto t^{-3/4} t^3 \propto t^{9/4} \quad (8)$$

Although in this case the particle number increases more rapidly than t^2 , we will not speculate whether the difference in exponent between 2 and 9/4 is sufficiently significant to require particle destruction!

Next, let us consider the relationship between age and temperature in cosmologies based on the LNH. For such cosmologies the variation of t with T is much more rapid than in conventional cosmologies (compare with equations (4) and (5))

$$\begin{aligned} \text{MD} : T &\propto n^{1/3} \propto \rho^{1/3} \propto t^{-1/3} \\ \text{RD} : T &\propto n^{1/3} \propto \rho^{1/4} \propto t^{-1/4} \end{aligned} \quad (9)$$

As before, if account is taken of the relative periods of time

that the Universe is MD and RD, we obtain

$$\begin{aligned} \text{MD} : t &\simeq 10^{18.5} T^{-3} \text{ s} \\ \text{RD} : t &\simeq 10^{23} T^{-4} \text{ s} \end{aligned} \quad (10)$$

These extremely strong dependences of t on T have several unfortunate consequences. We noted earlier that primordial nucleosynthesis occurs when $10^8 \lesssim T/T_0 \lesssim 10^9$. It follows from equation (10) that the age of the Universe is only $\sim 10^{-15}$ s when $T \simeq 3 \times 10^9$ K and, by $t \simeq 10^{-11}$ s the temperature has dropped to $T \simeq 3 \times 10^8$ K. Clearly there is no time available for any nuclear reactions to proceed. There is apparently no nucleosynthesis at all in cosmologies based on the LNH. In such models, then, the observed large and uniform abundance of helium⁶ is without explanation.

Another unpleasant aspect of the rapid variation of t with T is the extremely steep decline in the number of particles in the Universe with increasing temperature

$$\begin{aligned} \text{MD} : N &\simeq 10^{78} (T_0/T)^4 \\ \text{RD} : N &\simeq 10^{90} (T_0/T)^9 \end{aligned} \quad (11)$$

As a result, for $T \gtrsim 10^{10} T_0$ there is less than one particle in the Universe! Clearly, the usual assumptions of homogeneity will have broken down.

Finally, consider the scattering of photons by electrons in the early Universe. The number of scatterings per s is

$$\tau_\gamma^{-1} = n\sigma_{\gamma e} \simeq 10^{-20} T^3 \text{ s}^{-1} \quad (12)$$

Comparing equations (10) and (12) it follows that the total number of scatterings is $t/\tau_\gamma \ll 1$. Thus, there is no way to establish thermodynamic equilibrium between the photons and electrons and so the black-body spectrum of the background radiation is without explanation.

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Gravitational analogue of magnetic force

THE gravitational analogue of the magnetic force has been discussed^{1,2} within the framework of special relativity. The difference of opinion between these authors stems basically from the fact that a rigorous calculation of this force must be carried out using the general theory of relativity, and we give here such a calculation.

Consider two massive charged particles with masses, charges, coordinates and velocities, denoted by m_i , e_i , r_i and v_i , respectively ($i = 1, 2$). Then, to order c^{-2} , the Lagrangian for the system is³

$$L = L_0 - V \equiv L_0 - (V_{\text{EIH}} + V_{\text{D}} + V_{\text{M}}) \quad (1)$$

where $L_0 - V_{\text{EIH}}$ is the Einstein-Infeld-Hoffmann Lagrangian for uncharged particles⁴, $L_0 - V_{\text{D}}$ is the Darwin Lagrangian of classical electrodynamics for a system of two charges (see ref. 4, p. 168) and V_{M} consists of mixed mass-charge terms.

Explicitly, we have

$$L_0 = \frac{1}{2}m_1v_1^2 + \frac{1}{2}m_2v_2^2 + \frac{1}{8c^2}(m_1v_1^4 + m_2v_2^4) \quad (2)$$

$$-V_{\text{EIH}} = \frac{Gm_1m_2}{r} - \frac{Gm_1m_2}{2rc^2}[\mathbf{v}_1 \cdot \mathbf{v}_2 + (\mathbf{v}_1 \cdot \mathbf{n})(\mathbf{v}_2 \cdot \mathbf{n}) - 3(\mathbf{v}_1 - \mathbf{v}_2)^2] - \frac{G^2m_1m_2(m_1+m_2)}{2r^2c^2} \quad (3)$$

$$-V_D = -\frac{e_1e_2}{r} + \frac{e_1e_2}{2rc^2}[\mathbf{v}_1 \cdot \mathbf{v}_2 + (\mathbf{v}_1 \cdot \mathbf{n})(\mathbf{v}_2 \cdot \mathbf{n})] \quad (4)$$

and

$$-V_M = \frac{G}{2r^2c^2}[2e_1e_2(m_1+m_2) - (e_1^2m_2 + e_2^2m_1)] \quad (5)$$

where $\mathbf{n}$ is a unit vector along $\mathbf{r}_1 - \mathbf{r}_2$.

The existence of a gravitational analogue of the magnetic force (the Gv_1v_2 terms) is now apparent. It is instructive, however, to consider, as in refs 1 and 2, the two particles to be in equilibrium in their rest frames, from the balance of Newtonian and Coulomb forces. Then, we consider a frame of reference in which the particles, while maintaining the same distance apart, are moving with uniform velocity $\mathbf{v}_1 = \mathbf{v}_2 \equiv \mathbf{v}$ with $\mathbf{v}_1 \cdot \mathbf{n} = \mathbf{v}_2 \cdot \mathbf{n} = 0$. Thus

$$L_0 = \frac{1}{2}(m_1+m_2)v^2 + \frac{v^4}{8c^2}(m_1+m_2) \quad (6)$$

$$-V_{\text{EIH}} = \frac{Gm_1m_2}{r} \left(1 - \frac{v^2}{2c^2}\right) - \frac{G^2m_1m_2(m_1+m_2)}{2r^2c^2} \quad (7)$$

and

$$-V_D = -\frac{e_1e_2}{r} \left(1 - \frac{v^2}{2c^2}\right) \quad (8)$$

The corresponding 'generalised force' on particle 1 from particle 2 is

$$F = \left| \left(\frac{\partial L}{\partial \mathbf{r}_1} \right) \right| = -\frac{Gm_1m_2}{r^2} \left(1 - \frac{v^2}{2c^2}\right) + \frac{G^2m_1m_2(m_1+m_2)}{r^3c^2} + \frac{e_1e_2}{r^2} \left(1 - \frac{v^2}{2c^2}\right) - \frac{G}{r^3c^2} [2e_1e_2(m_1+m_2) - (e_1^2m_2 + e_2^2m_1)] \quad (9)$$

Thus, the condition for equilibrium in the moving frame is $e_i = \pm G^{1/2}m_i$. In other words, as previously noted^{5,6} we have the same condition for equilibrium in the general relativistic case as in the Newtonian case. In addition, we notice that the gravitational analogue of the magnetic force, in this particular example, is simply obtained from the magnetic force $-e_1e_2v^2/2r^2c^2$ by the replacement $e_1e_2 \rightarrow -Gm_1m_2$ and, to lowest order in (v/c) , this agrees with the result given in ref. 1. On examination of equations (3) and (4), however, it is clear that this is not true in general.

In fact, it is now clear that arguments based on the use of special relativity only^{1,2} are inadequate in two major respects: (1) They fail to uncover the existence of various higher-order terms (in addition to the Gv^2 terms, the so-called gyron force¹ terms), such as the purely gravitational G^2 terms (which, at least for bound-state problems—perihelion precession of

Mercury—are comparable in magnitude to the Gv^2 terms), nor the mixed Ge_ie_j terms. The existence of these nonlinear terms is intimately related to a well-known facet of general relativity theory—that the electromagnetic field and the gravitational field itself both act as sources of the gravitational field. (2) They provide no unambiguous way of deciding on the correct law of transformation of forces, whereas such a transformation emerges naturally in the general relativistic approach.

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Dispersion and the transverse aether drag

THERE has been controversy about the correct expression of the displacement, d , introduced by the transverse motion of a dispersive dielectric slab on a light beam. Player¹ proposed the formula $d = vt(n_g - n^{-1})/c$, where v is the slab transverse velocity, t the slab thickness, n_g and n the group and wave refractive indices of the medium, respectively, and c the velocity of light in free space. This expression has been verified in very careful experiments reported by Jones². I point out here that a simple model demonstrates that (in agreement with Player's formula) d must vanish when $v_g v_\phi = c^2$, where $v_g \equiv c/n_g$ is the group velocity and $v_\phi \equiv c/n$ is the phase velocity in the slab.

Let the slab be cut out from a set of plane parallel perfectly conducting surfaces. In other words, instead of considering a real dielectric, we consider a set of perfectly conducting sheets in air. Such artificial dielectrics are commonly used at microwave frequencies. The direction of the incident ray, the direction of the incident polarisation ($\mathbf{E}$ field), and the direction of the motion are assumed to be in the plane of these surfaces (see Fig. 1). The propagation of electromagnetic waves in the artificial dielectric slab just described is isotropic in the plane of the surfaces and satisfies the well known relationship $v_g v_\phi = c^2$. On the other hand, the motion of perfectly conducting surfaces in their own plane is immaterial. Therefore the transverse displacement d of the light beam must be equal to zero when the relation $v_g v_\phi = c^2$ holds.

The same conclusion can be reached by considering a particle with non-zero rest mass, such as an electron, traversing a set of perfectly transparent grids at different potentials³. As is well known, the de Broglie matter wave associated with the electron satisfies the relationship $v_g v_\phi = c^2$, where the group velocity v_g can be identified with the electron velocity. Here

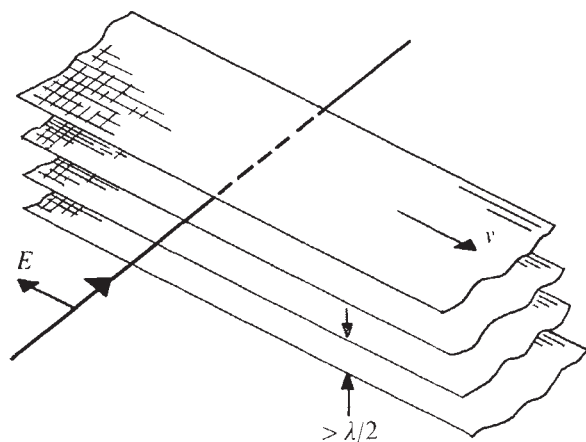


Fig. 1 Gedanken apparatus, consisting of plane parallel conducting sheets moving (velocity v) transverse to incident radiation.

again, it can be seen that a motion of the grids in their own plane is immaterial and should not affect the electron trajectory.

My arguments, of course, do not prove Player's formula, but they prove that alternative general formula that do not satisfy the requirement set up above are incorrect. Note that the condition $d=0$ when $v_g v_0 \rightarrow c^2$ should hold also for light beams at non-normal incidence, a case that was not considered explicitly by Player.

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Tin mineralisation and mantle hot spot activity in south-eastern Missouri

SILLITOE has pointed out interesting relationships between tectonic setting and a particular class of tin mineralisation¹. Typical mineralisation in these deposits comprises disseminated cassiterite accompanied by topaz, lithia mica, and wolframite in greisens and quartz veins above small subvolcanic alkaline and peralkaline granite plutons which lack demonstrable affiliation with contemporary plate boundaries.

I here describe briefly the tin mineralisation and petrologic-tectonic setting of the Saint Francois Mountains volcanic plutonic terrain of south-eastern Missouri, which may possibly represent the oldest example of this class of mineral deposit.

The Saint Francois Mountains form a distinctive unmetamorphosed igneous complex comprising chiefly alkalic to peralkalic rhyolite and granite, associated with a major negative gravity anomaly². The plutons seem to be more or less concordant injections into chemically-related rhyolitic ignimbrite and breccia sheets³. The date of this volcanic-plutonic episode is well established by recent detailed geochronologic studies⁴ at about 1,500 Myr BP. The effusive rocks range in composition from andesite to rhyolite, whereas the plutonic rocks vary in composition from granodiorite to granite and minor syenite. The volcanic series and the plutonic series are each volumetrically dominated by the high-silica end members³. Porphyritic textures are widespread in many of the intrusive bodies; the

granites locally display the well developed rapakivi texture⁵ characteristic of anorogenic granites throughout the world⁶. Palaeozoic strata cover the country rock which surrounds the igneous exposures of the Saint Francois Mountains, but drill-core studies indicate the 1,500-Myr-old rocks were intruded into and erupted on an older metamorphic-plutonic terrain⁷. The exposed volcanic-plutonic complex is cut by diabase dykes and layered gabbro sills of later, but uncertain, Precambrian age⁸.

The Precambrian volcanic rocks of south-eastern Missouri have long been known for their association with magmatic iron ores⁹⁻¹¹. Tin mineralisation of subeconomic grade, however, is associated with the contact between rhyolite roof rocks and the intruding granite at a locality known as the Silver Mine (90.5°W; 37.5°N). The roof-zone greisen deposits of that locality have been sporadically worked for silver and tungsten during the past 100 yr. Mineralisation in the Silver Mine area consists of well-defined quartz veins containing disseminated cassiterite, lithia mica (zinnwaldite?), topaz, fluorite, wolframite (hubnerite), arsenopyrite, pyrite, chalcopyrite, sphalerite, and argentiferous galena¹². Major, trace, and rare earth element distributions in the veins and altered wallrocks indicate extensive replacement of the host granite by high temperature, fluorine-rich solutions¹³. Original feldspars in the wall-rock adjacent to the veins have been completely destroyed and supplanted by an assemblage of topaz, quartz, fluorite, sericite, lithia mica, and cassiterite¹³. Beryl and tourmaline are not present in the veins or the well defined vein envelopes of sericitic wallrock alteration¹³. This mineral assemblage and its petrologic setting are remarkably similar to those of the Younger Tin Fields of Nigeria¹⁴ and the tin deposits of Rondônia, Brazil¹⁵.

The tin mineralisation in south-eastern Missouri differs in major ways from the well known tin deposits of Bolivia and Japan. The latter are both located in calc-alkaline igneous settings and are thus apparently related to convergent plate processes. Sillitoe *et al.*¹⁶ have described the Bolivian tin deposits as porphyry type mineralisation characterised by pervasive sericitic alteration, stockworks of veinlets, and widespread hydrothermal intrusion breccias—features entirely lacking in the Saint Francois Mountains deposit. The tin mineralisation of Missouri bears a resemblance to the subvolcanic (xenothermal) deposits of Japan¹⁷ which also contain topaz and lack both tourmaline and beryl. The Japanese ores, however, contain tin minerals other than cassiterite (for instance, stannite, stannoidite, and mawsonite), primary bornite and chalcocite, native silver, and silver sulphosalts in a gangue which includes apatite, rhodochrosite, and orthoclase¹⁷. None of these minerals has been reported at the Silver Mine workings¹². Lithia mica is present in Japanese hypothermal tin veins but is not found in the subvolcanic (xenothermal) deposits¹⁷. In the Andes of South America, subduction processes have resulted in a definite zoning of metal provinces wherein iron mineralisation and tin-molybdenum mineralisation are widely separated in space¹⁸. The spatial coincidence of south-eastern Missouri iron and tin mineralisation does not support the hypothesis that there is a former subduction zone beneath the Precambrian igneous terrain of the region.

The hypothesis that tin-bearing alkaline igneous complexes are generated above mantle hot spots before the formation of triple junctions and associated rifts¹ is supported by geological and geophysical relationships in south-eastern Missouri. Burke and Dewey¹⁹ have interpreted the Mississippi Embayment as a failed arm (aulacogen) of a late Palaeozoic triple junction centred near Jackson, Mississippi, but there is evidence that this event was preceded by a major period of late Precambrian rifting in central North America which commenced about 1,300 Myr BP (refs 20-22).

Tin-bearing subvolcanic complexes in Nigeria, Rondônia, and Namibia occur in elongated belts 300-400 km long¹.

Deep drilling of the Mid-continent Precambrian terrain has returned rock samples which are similar in petrography and general age to those exposed in the Saint Francois Mountains. These samples apparently represent buried subvolcanic complexes, similar to those exposed in south-eastern Missouri, which are arranged in a north-east-south-west trending belt¹. Data from Muehlberger *et al.*²³ suggest that the age of the complexes becomes progressively younger south-westward, inviting the speculation that the belt records a period of north-eastward plate movement over a mantle hot spot which supplied tin, fluorine, and other mantle-derived elements to rising magmas. It must be noted however, that the only tin deposit at present known to exist in this belt of subvolcanic complexes is that exposed in the Saint Francois Mountains.

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incompressibility defined by the equation

$$\frac{1}{k_s} = \frac{1}{\rho} \left[\frac{\partial \rho}{\partial p} \right]_s$$

The parameter

$$\phi = \frac{k_s}{\rho} = V_p^2 - \frac{4}{3} V_s^2 \quad (2)$$

where V_p and V_s are the velocities of P and S waves and are known from seismology. This distribution of density is neutrally stable since the change from the adiabatic expansion suffered by an element of fluid displaced radially outward is exactly equal to the change in density of the surrounding fluid over this displacement.

Suppose now that the density distribution depends not only on adiabatic compression but also on temperature (or entropy). Then, for a homogeneous medium

$$\frac{d\rho}{dr} = \left[\frac{\partial \rho}{\partial p} \right]_s \frac{dp}{dr} + \left[\frac{\partial \rho}{\partial s} \right]_p \frac{ds}{dr} \quad (3)$$

The first term on the right hand side is the Adams-Williamson density gradient and the second the contribution from the change in density with entropy. If the entropy is constant throughout the fluid so that $ds/dr = 0$, equation (3) reduces to equation (1) and the density gradient is uniquely determined. This, however, is very unlikely since it implies that the temperature gradient dT/dr is exactly equal to the adiabatic gradient $g\alpha T/c_p$ throughout the core since

$$\left[\frac{\partial \rho}{\partial s} \right]_p \frac{ds}{dr} = \alpha \rho \left[-\frac{dT}{dr} - \frac{g\alpha T}{c_p} \right] \quad (4)$$

where α is the volume coefficient of expansion and c_p the specific heat at constant pressure.

Birch³ has written equation (3) in the alternative form

$$\frac{d\rho}{dr} = \frac{-g\rho}{\phi} + \alpha\rho\tau \quad (5)$$

where τ is the excess of the actual temperature gradient over the adiabatic gradient. A necessary condition for convection to be possible in any stationary layer is that $\tau > 0$. Various estimates of τ have been made in the past with differing conclusions.

An estimate of the term $\alpha\rho\tau$ in the Earth's core may, in principle, be obtained from an independent evaluation of the actual density gradient and that given by the Adams-Williamson equation. Such an approach is inherent in the work of Pekeris and Accad⁴ who wrote

$$\alpha\rho\tau = \frac{g\rho\beta}{\phi} \quad (6)$$

so that

$$\beta = \beta(r) = 1 + \frac{\phi}{g\rho} \frac{d\rho}{dr} \quad (7)$$

They chose constant values of β (-0.2, 0 and 0.2) throughout the core and compared the observed free oscillation periods of the Earth with those calculated from their estimate of β . A programme to try to determine the behaviour of β in the Earth's core has been undertaken. Preliminary calculations show that the extreme values ± 0.2 are physically unrealistic and that $|\beta|$ must be much smaller. In some models of the Earth, however, the density stratification changes from stable ($\beta < 0$) in one part of the core to unstable ($\beta > 0$) in another.

The stability of the earth's core and the geodynamo

RECENT work^{1,2} has indicated that precession fails by at least an order of magnitude to provide the power necessary to drive the geodynamo. It is therefore necessary to consider again thermal convection in the Earth's core, which we do in this paper.

Thermal convection requires that the fluid core be unstable in the radial direction. A fluid element displaced radially from the Earth's centre must find itself surrounded by fluid of greater density so that it will continue to rise. If this is not the case the fluid element will either remain in its new position (neutral stability) or suffer a restoring force towards its initial position. The density distribution $\rho = \rho(r)$ which determines the stability of the core will depend on two thermodynamic variables such as pressure p and entropy s , together with as many parameters as there are components of the core fluid. In any homogeneous region in which changes of density are due to adiabatic compression alone, the density distribution is given by the Adams-Williamson equation

$$\frac{d\rho}{dr} = \frac{-g\rho^2}{k_s} = \frac{-g\rho}{\phi} \quad (1)$$

where g is the acceleration due to gravity and k_s the adiabatic

It can be seen that β may be estimated (from equation (7)) throughout the outer core without any knowledge of the temperature gradient. It is very sensitive, however, to the variation of the density gradient $\frac{d\rho}{dr}$. Crossley (personal communication)

has estimated β for a number of Earth models, and obtained values lying in general between about -0.2 and 0.2 , although some models gave values outside this range over part of the core. Knowing β , the excess τ of the actual temperature gradient over the adiabatic temperature gradient may be estimated from equation (6). Estimates of τ are more unreliable because of uncertainties in the values of α in addition to those in β . Frazer⁵ has examined the literature and found that estimates of α range from 3.6×10^{-6} to $10 \times 10^{-6} \text{ K}^{-1}$. It is likely that α will be pressure dependent and Jacobs⁶ suggested that there may be a linear relationship between $1/\alpha$ and pressure in analogy with Bullen's⁷ compressibility-pressure hypothesis. He obtained values of α of $9.3 \times 10^{-6} \text{ K}^{-1}$ at the core-mantle boundary and $4.4 \times 10^{-6} \text{ K}^{-1}$ at the inner core-outer core boundary—both these values of α lie within the range of those given by Frazer.

The adiabatic gradient may, alternatively, be written in terms of Grüneisen's 'constant' γ

$$\frac{dT}{dr} = \frac{-g\alpha T}{c_p} = \frac{-g\gamma T}{\phi} \quad (8)$$

γ was originally defined for solids and its extension to liquids has been questioned⁸. It can be seen from equation (8) that estimates of the adiabatic gradient are further hampered by uncertainties in the variation of c_p (or γ). When values of the various parameters are assigned, the adiabatic gradient in the core may be estimated once a temperature has been 'fixed' at any point.

Write

$$X = \frac{\text{Excess temperature gradient}}{\text{Adiabatic temperature gradient}} = \frac{\beta c_p}{\alpha^2 \phi T} = \frac{\beta}{\alpha \gamma T} \quad (9)$$

Taking extreme values of the parameters ($T = 5,000 \text{ K}$, $\alpha = 10^{-6} \text{ K}^{-1}$, $\gamma = 1.75$), the minimum value of $X \approx 11\beta$. Hence even if $|\beta|$ is as small as 0.1 , the excess temperature gradient is equal to the adiabatic. It would thus seem that $|\beta|$ is unlikely to exceed ~ 0.01 . This is a significant constraint on any future study of core dynamics. Dziewonski *et al.*⁹ independently concluded that densities and velocities in the core (and lower mantle) are consistent with the Adams-Williamson equation ($\beta = 0$) to $< 0.2\%$. It is not yet certain whether additional constraints on β will allow us to determine the gross stability structure of the outer core. In the meantime, it is interesting to examine the properties of some models of the Earth that have been proposed. For Model PEM-A, $\beta > 0$ throughout the core, thus permitting convection. For other models (1066A, 1066B) β is positive over most of the outer core, although becoming negative over $\sim$ the middle 700 km. Values of β are so large for these models that the corresponding values of τ lead to highly super-adiabatic gradients near both boundaries of the outer core.

If the outer core does have regions of instability adjacent to its boundaries with the mantle and inner core, separated by a stable central region, it is interesting to speculate whether convection in either of the unstable regions could power the geodynamo. Verosub¹⁰ recently suggested that the Earth's magnetic field was the sum of two oppositely directed dipoles and was able to account for reversals of the field without requiring a self-reversing dynamo. He suggested that the two sources are located in the inner and outer cores. The source field in the liquid outer core could be generated by magneto-hydrodynamic action, but it is much more difficult to postulate a source mechanism in the solid, inner core. It is perhaps

possible that at the pressures and temperatures existing in the inner core, new solid state phenomena exist which could lead to permanent magnetisation. On the other hand, it is possible that both sources are in the outer core in two different unstable regions as indicated above. Such a possibility would also be compatible with Kono's¹¹ model of the Earth's magnetic field in which he postulated ten dipoles in the core with equal moments and directions either parallel or anti-parallel to the rotation axis. It is interesting to note that Gubbins (unpublished) has shown that, contrary to expectation, the absence of convection, or at least sufficiently vigorous convection, in the outer regions of the core does not inhibit the generation of magnetic field. Specifically, he has shown that circulation on a global scale surrounded by a stable, electrically conducting region is more efficient for magnetic field generation than fluid motion throughout the entire outer core.

It must be emphasised that the arguments given in this note have assumed a homogeneous core of one component only. Thus, in addition to the entropy term in equation (5) for the density gradient we must add terms for additional light alloying components such as sulphur or silicon to obtain a more realistic model of the Earth's core. The relative importance of non-adiabatic conditions and inhomogeneities are being investigated.

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The 20-yr oscillation in eastern North American temperature records

It has been argued (see for example ref. 1) that there is little or no reliable evidence for 11- or 22-yr cycles in meteorological time series, and that even if a significant spectral peak exists, the identified cycle may not maintain its phase and amplitude sufficiently to allow reliable prediction. To investigate this we have examined January temperature records for 19 stations in eastern North America. Our results show that: first, a pervasive 20-yr peak exists in the spectra of January temperatures in eastern Canada and the United States; secondly, the 20-yr oscillation was largely in phase over this region until approximately 1960; and thirdly, the predictability of this oscillation is, at best, only marginal.

With regard to spectral analysis the major problem of modern instrumental data is their availability only over times short by comparison with some of the interesting low frequency components. Maximum entropy spectral analysis (MESA) and filtering procedures now make possible, however, much more precise and reliable analysis. Recently, for example, MESA has provided convincing evidence of a 10.5-yr peak in the spectra of annual temperatures for selected North American stations, although no analysis of predictability was made².

For each record examined here, the Burg algorithm (Burg, unpublished, but see refs 3 and 4 for computational procedures) was used to estimate a 31-lag autocorrelation function from which the ME spectrum was calculated. To examine the pre-

Table 1 Stations used in this study. For ease of comparison all variances of bandpass filtered data have been normalised to percentage of observed variance

Station	Years of record	Observed variance ($^{\circ}\text{C}$) ²	Bandpass variance (%)	75% Lower limit bandpass variance (%)*
1 Sable Island	1898–1974	2.09	14.5	10.9
2 Charlottetown	1889–1974	5.28	8.7	6.5
3 Eastport	1874–1975	4.76	10.8	8.2
4 Montreal	1875–1974	7.09	9.9	7.5
5 Hanover	1835–1975	7.69	9.7	7.3
6 Toronto	1841–1974	7.19	7.1	5.4†
7 Blue Hill	1832–1975	5.82	10.2	7.7
8 New Haven	1779–1975	5.17	8.5	6.4
9 New York	1869–1975	6.08	12.5	9.4
10 Pittsburgh	1875–1975	7.98	7.4	5.6†
11 Columbus	1879–1975	9.19	7.7	5.8†
12 Washington	1873–1975	6.74	12.3	9.3
13 Lynchburg	1872–1975	6.09	8.2	6.2†
14 Cape Hatteras	1875–1975	6.37	12.6	9.5
15 Charleston	1823–1975	6.04	11.9	9.0
16 Columbia	1888–1975	6.23	12.5	9.5
17 Mobile	1873–1975	6.55	13.3	10.0
18 Memphis	1872–1975	7.58	8.6	8.6
19 Nashville	1873–1975	7.88	9.1	6.9

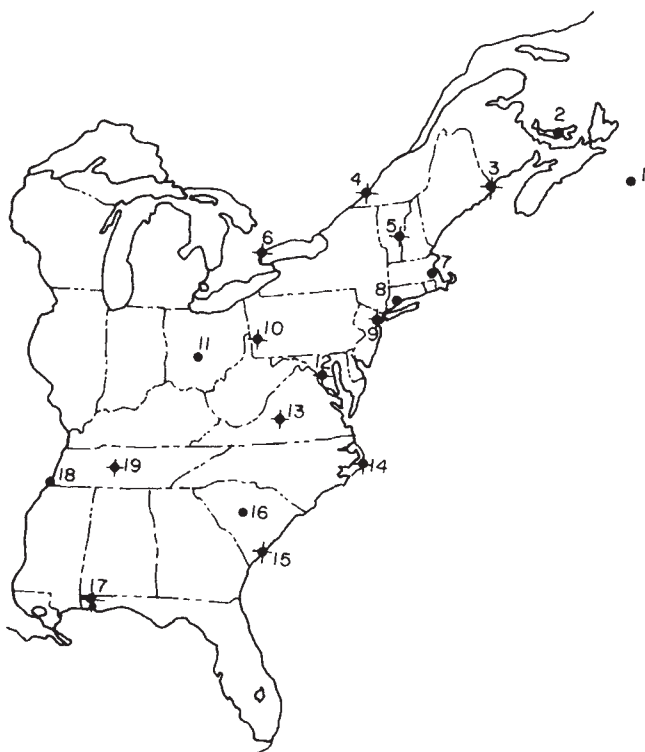
*Expected variance bandpassed: 6.3% (white noise).

†Overlaps white noise range.

dictability of the meteorological records we used the ME optimal filtering approach^{4,5}. The computational procedure consists essentially of characterising a time series by an autoregressive process, the basic idea being that ME supplies a unique way of choosing unknown autocorrelation values to infinite lag (given a finite autocorrelation). These are chosen such that no information or entropy is added

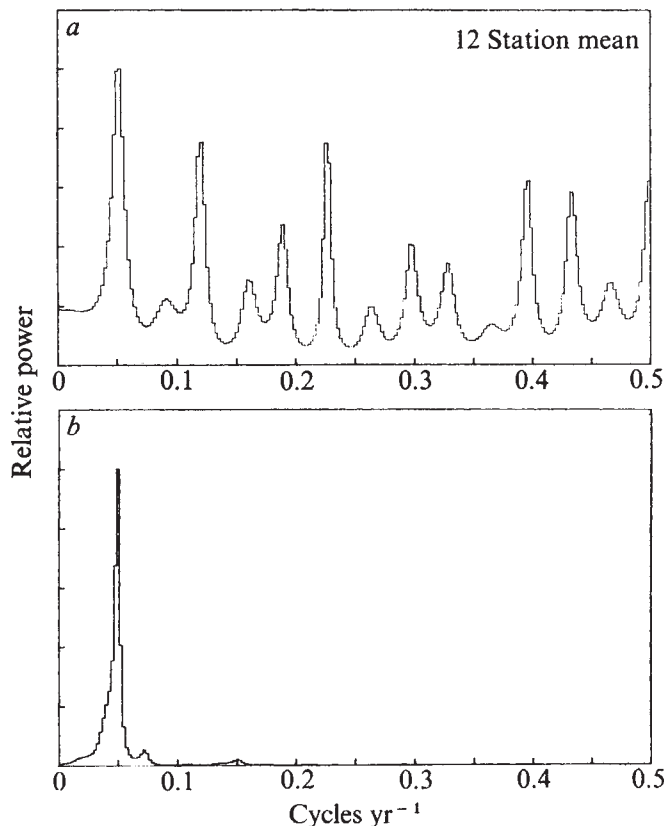
$$\frac{\partial h}{\partial \phi(k)} = 0 \quad |k| \geq N+1$$

where h is entropy, $\phi(k)$ is the autocorrelation at lag k , and N is the maximum lag number. Consequently, the optimal prediction

Fig. 1 Location of the 19 stations used in this study. Numbers refer to Table 1. Stations shown with + used to form regional temperature time series.

filter for arbitrary distance may be determined by MESA. Since records can be extended into the past and future, filtering can be accomplished without loss of endpoints in the real data domain.

We used the 19 stations shown in Table 1 and Fig. 1. All data were taken from the World Weather Records supplemented by

**Fig. 2** *a*, Maximum entropy spectrum of regional January temperature time series. The 12 stations indicated + in Fig. 1 were used for the time period 1875–1974. *b*, Maximum entropy spectrum of double solar cycle time series.

data published by the Canadian and US Weather Services. For one of the stations (Hanover, New Hampshire) we calculated the ME spectra of each of the four seasons and of each month. A 20-yr peak was prominent only in the winter spectrum, and, of the winter months, only in January. It is perhaps significant that this is when the Northern Hemisphere

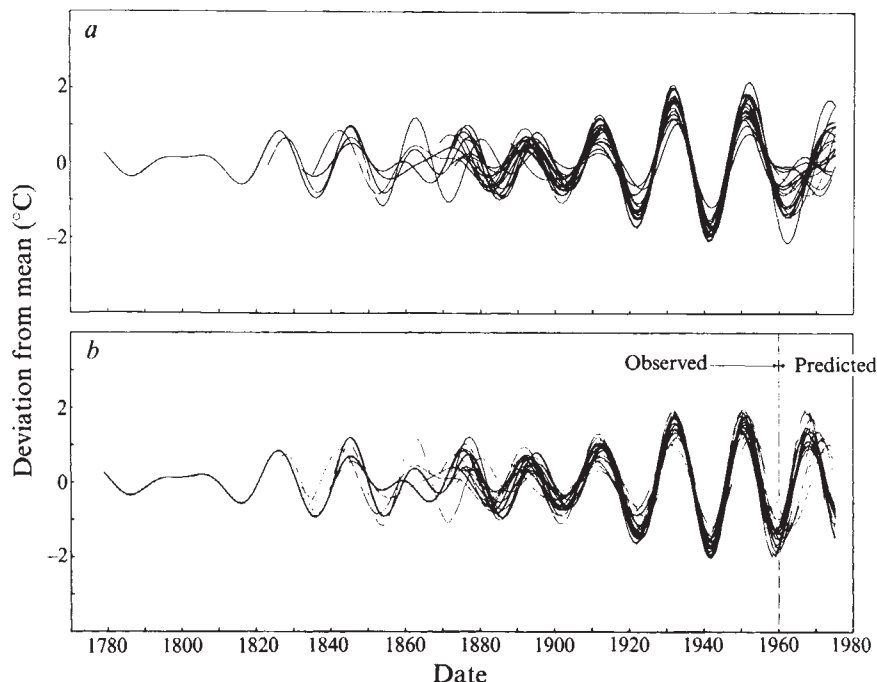


Fig. 3 *a*, Bandpass filtered January temperatures. *b*, Same as *a* but shows optimal prediction 1960–75.

circulation is most vigorous and the northward transfer of heat by large scale eddy activity is at its maximum⁶.

In addition to calculating the ME spectrum for each record we performed the following operations on this set of stations: (1) Formed a regional January temperature time series using the 12 stations indicated in Fig. 1 for the period 1875–1974 and calculated the ME spectrum of this regional series (Fig. 2*a*). (2) Optimally extended each record and then isolated the 20-yr oscillation by bandpass filtering (Fig. 3*a*). (3) Truncated the data at 1959 and then performed the same optimal extension and filtering as shown in Fig. 3*b*.

The salient feature of each of the individual ME spectra of January temperatures was the prominent peak at ~ 20 yr. The spectra of the regional temperature series (Fig. 2) indicate that the 20-yr oscillation has been substantially in phase over the region. The finite width of the peak, however, is indicative of some drifting of phase and variation in amplitude—what we term a stochastic rather than a deterministic process.

Figure 2*b* shows the ME spectrum of the double solar cycle calculated from data given by Chernosky and Jose^{7,8}. The coincidence of the 20-yr peak in this spectrum with the 20-yr peak of the temperatures is striking. We emphasise, however, that this coincidence does not imply that correlation exists between the two phenomena, let alone any causality, but to attribute this coincidence solely to chance seems quite unjustified.

Figure 3*a* shows the bandpass filtered records for all 19 stations. The relevant points are: (1) the near synchronism and stability of phase relationships from about 1890 until the late 1950s, and (2) their absence before 1890 and after 1960. Figure 3*b* illustrates the danger of simple projection as a means of prediction. Although there is some phase drift in the predicted records, the chaotic behaviour of the data would have obviated any regional prediction.

To provide a statistical measure of the significance of the spectral components isolated by the bandpass filter, we calculated their variance and the lower 75% confidence limit using the χ^2 distribution for comparison with the expected variance of bandpassed white noise in this same frequency range. In only four instances did this limit include the expected value of white noise.

In conclusion we feel the results presented here demonstrate a pervasive 20-yr oscillation in January mean temperatures over a substantial portion of eastern North America. This, coupled with Currie's² demonstration of a 10.5-yr spectral peak,

illustrates the resolution inherent in MESA when dealing with the generally short time scales of instrumental meteorological data, a resolving power generally unavailable at the time of Monin and Vulis's¹ review.

In addition, optimal extension and bandpass filtering have shown that the sample stations were oscillating substantially in phase from about 1890 to about 1960. Comparison of the optimally extended data from 1960 on with the actual data clearly demonstrates the inherent danger of predicting quasi-periodic phenomena on the basis of simple projection.

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The hydration of Portland Cement

ALTHOUGH extensive X-ray data have been recorded, until relatively recently¹ the microstructural changes which attend the hydration of Portland Cement have received scant attention. We here report electron transmission observations of the reaction and draw attention to analogous silicate models which suggest a probable mechanism.

Scanning electron microscopy of cement specimens which have been dried for various periods after addition of water to di- or tri-calcium silicate mixtures (Portland Cement) show that there are two stages in the reaction involving (1) the formation of an initial coating of gelatinous hydrate (conventionally referred to as calcium silicate hydrate gel) on the anhydrous crystals, and (2) subsequent growth from this coating of fine fibrillar material, the interlocking of which is thought to cause the cement to harden and develop a high compressive strength.

This picture is compatible with the results of calorimetric studies which show that the rates of heat evolution over periods of 10^2 – 10^3 h can be interpreted as resulting from a diffusion-controlled phase reaction proceeding by nucleation and growth.

Studies have been made in this laboratory by direct observations in transmission within a 'wet' environmental cell (2) placed in a 1-MeV electron microscope. Time lapse pictures, Fig. 1, confirm the above mentioned two-stage nature of the reaction, but also provide further detail of the fibrillar product, suggesting (Fig. 1c) that it is not solid crystalline material but is irregular and tubular in form. The product is not crystalline in a conventional sense and does not yield coherent diffraction

Fig. 1 Cement and water samples viewed in the environmental stage of the HVEM. *a*, after 3 h, showing the initial gel coating around a cement grain and small angular crystals of Portlandite (left). *b*, after 1 d, showing fibrillar development of C–S–H gel around the cement grains, illustrated in detail in *c*.

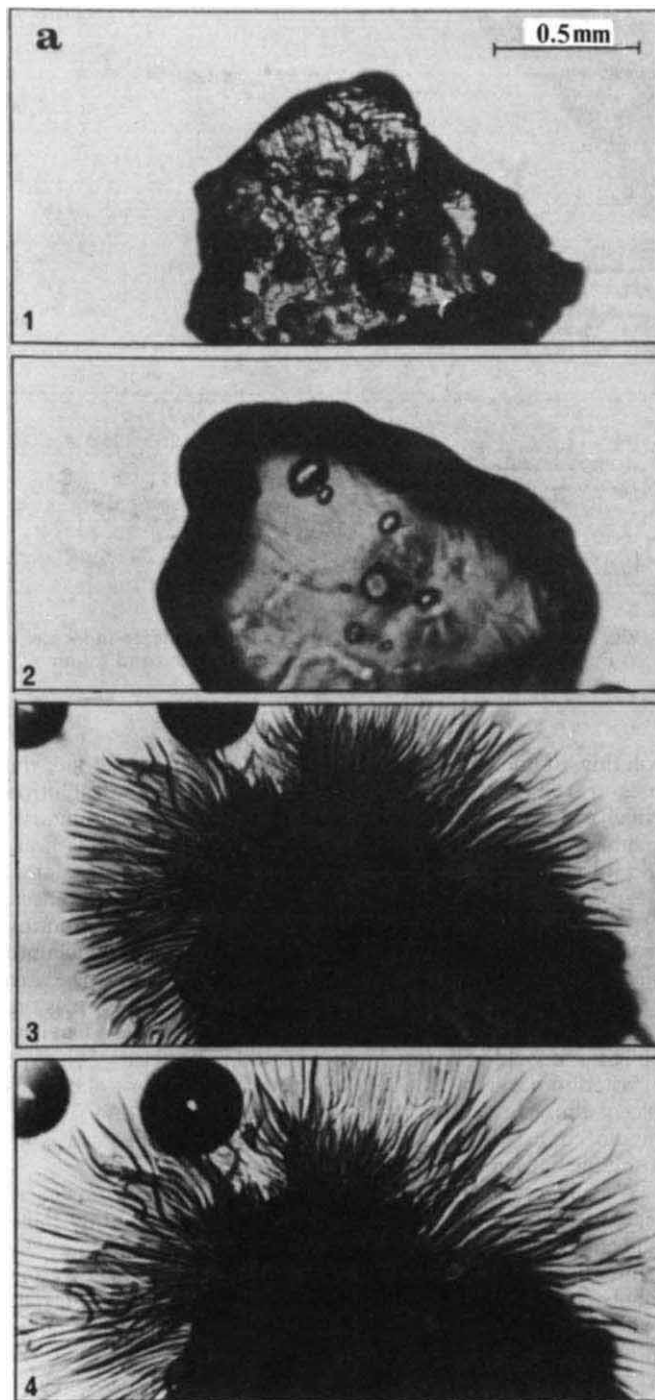
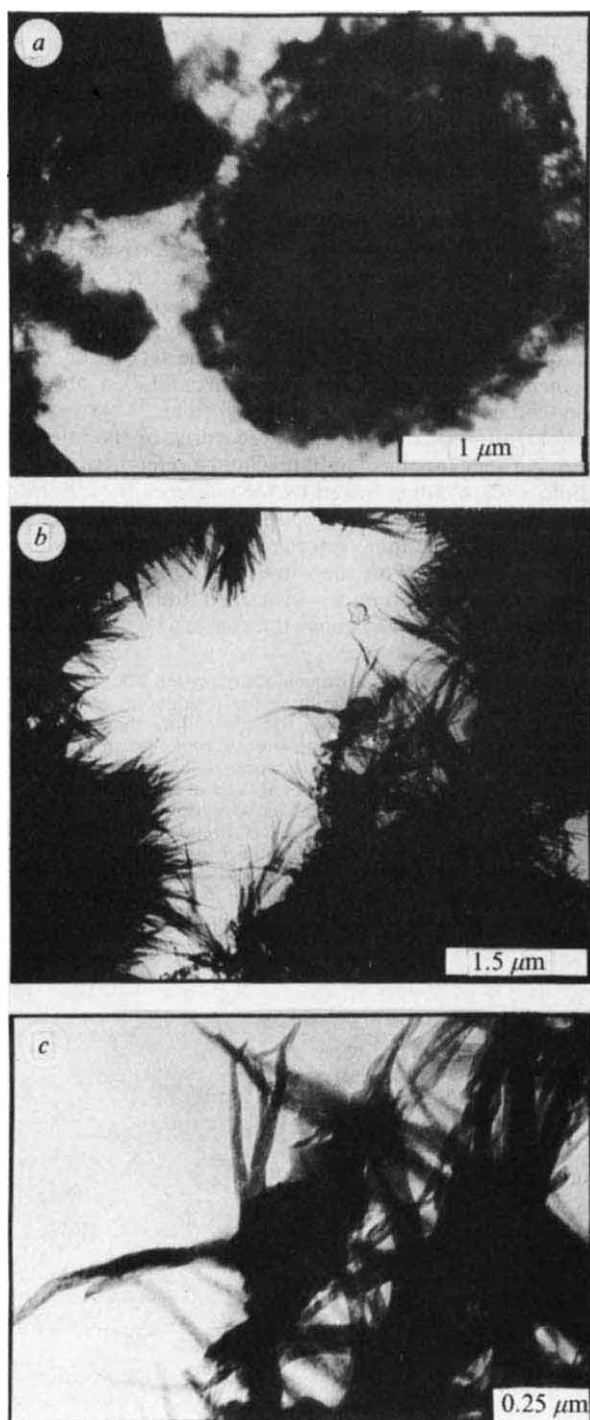


Fig. 2 Sequence showing the development of a 'silicate garden' from a crystal of $\text{Co}(\text{NO}_3)_2$ immersed in dilute sodium silicate solution. The time interval (1–4) is ~ 30 s. Optical micrograph.

patterns, and the driving forces which might lead to such growth, following an earlier incubation period might seem to be obscure.

The growth of hollow or tubular forms is, however, not a unique phenomenon, being characteristic of a wide range of compounds which occur in so-called 'silica gardens'. Figures 2a–d are time lapse optical micrographs of an aqueously soluble salt ($\text{Co}(\text{NO}_3)_2$) which has been brought into contact with sodium silicate solution, and although the rates of formation and scale are some 10^2 or 10^3 greater than those illustrated in Fig. 1—for Portland Cement—the striking analogy cannot be overlooked.

The mechanism by which the tubular 'silica gardens' form involves osmosis through the initial gelatinous coating which acts as a semi-permeable membrane, so that with continued

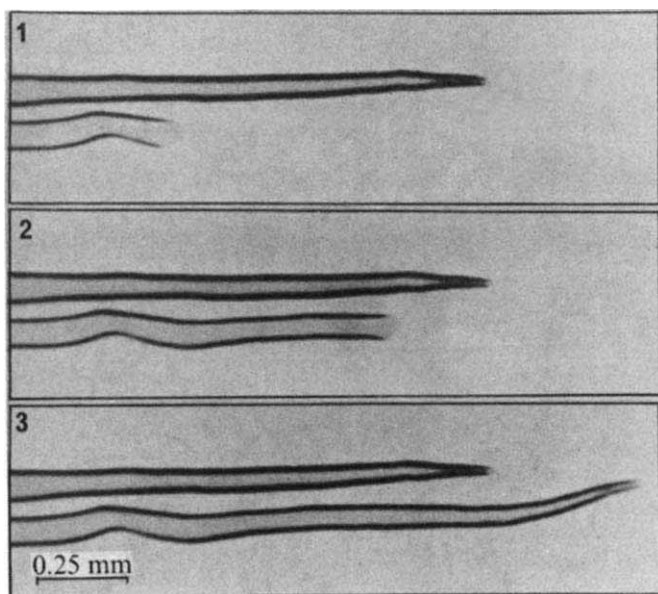


Fig. 3 10-s time lapse sequence of two cobalt silicate tubes (as in Fig. 2) illustrating in detail the tip reaction cone and terminal tapering effect. Optical micrographs.

solution of the salt crystal within the primary envelope, the pressure rises until rupture occurs locally. The jet of salt solution which erupts into the silicate solution precipitates continuously a tube of solid material, and as salt solution is pumped along the tube so formed—from the primary envelope—almost steady-state growth can proceed for considerable distances. Details of the growth (Fig. 3) show how a cone of salt solution reacts with silicate solution and builds up a solid wall until viscous drag and falling pressure limits the flow and leads to necking down or closing of the tubes. These silicate tubes are of variable section, are remarkably strong and brittle when dry, but do not yield coherent X-ray diffraction patterns. This type of reaction occurs with water soluble salts of any metal cations except those in Group 1A of the Periodic table, and further, should be possible whenever the initial reaction product is an insoluble but semi-permeable material.

We suggest that the analogy between the 'silica garden' reaction and that which occurs during the hydration of Portland Cement is close, and goes some way towards explaining why cement fibrils are not faceted solid crystals such as those commonly found during crystal growth from aqueous solution. The two-stage character of the reaction is then explicable in terms of a model for which osmosis provides the driving force for secondary growth, and although direct extension to include cement reaction products must involve a different chemical combination, the diffusion species in each case would be water and the initial coating a colloidal silicate gel.

The above outline necessarily raises many points of detail and speculation which require a more complete and discursive account, to be published elsewhere. Further work is in progress in this laboratory, the better to establish the tubular shape of Portland Cement hydration products, and to quantify the factors which control the growth of silica gardens, with the intention of confirming, or otherwise, the connection between these materials and the mechanism by which they grow. It is worth noting that silicate membranes are indeed semi-permeable (to water) and that the lengths and diameters of the tubes in any one system (for example CaCl_2 , $\text{Co}(\text{NO}_3)_2$, FeSO_4 , with sodium silicate) decrease with reaction rate, so that extrapolation to the slower growing and finer cement hydrates is both in the correct direction and corresponding scale.

Establishment of the reaction mechanism is a necessary step if this important process, of cement hardening, is to be understood and controlled.

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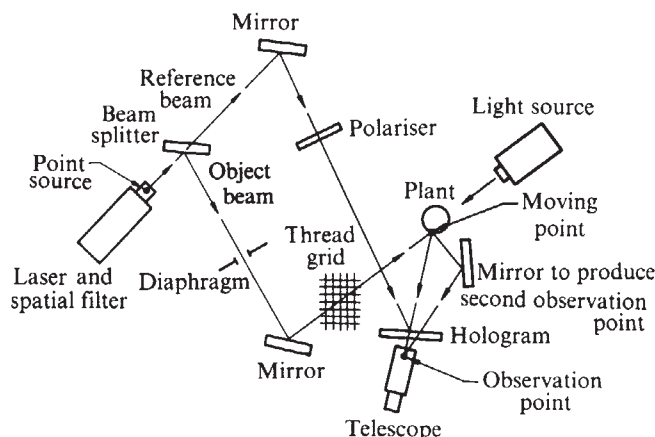
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Analysis of transient plant movements by holographic interferometry

HOLOGRAPHIC interferometry is a photographic technique which can be used to measure minute displacements of objects¹. Williams² has shown holographic interferograms of painted wheat shoots, but in his initial work there was no attempt to make quantitative measurements of either plant displacement or, more importantly, transient plant response. The present work demonstrates the potential of holographic interferometry in plant movement analysis by showing previously unobtainable data on the bending and short term transient response of a mature, slow moving plant. Our results suggest that the holographic interferometric technique may provide the means to measure accurately such important plant movement parameters as velocity and displacement with much higher resolution than is available from any other method. In addition, the technique substantially improves the time resolution of plant movement analysis, thus enabling more detailed observation of the tropic response as a function of time.

A hologram is a photographic recording of the interference pattern between an object light field and a reference field. When the hologram is reilluminated by the reference field, diffraction causes the object field to be reconstructed. A double exposure hologram or holographic interferogram³ is formed when two holograms are taken on the same photographic plate. When a double exposure hologram is visualised, the two reconstructed images interfere as if they coexist in space. The displacement, d ,

Fig. 1 The holographic arrangement consisted of a He-Ne laser with spatial filter, a beam splitter for producing the reference and object beams, two mirrors for controlling the beam directions, a polariser for controlling the reference beam intensity, a diaphragm for eliminating extraneous reflections, and a holographic camera. Additional elements useful for plant movement studies were a thread grid which cast a shadow grid on the plant, one to three mirrors for obtaining additional observation points, a telescope mounted on a sextant for measuring angles and counting fringes, and a monochromator for producing the phototropic stimuli. The components were positioned on a $2.5 \times 61 \times 100$ -cm steel plate using magnetic mounts. Vibration isolation was obtained by supporting the apparatus on four inflated rubber inner tubes.



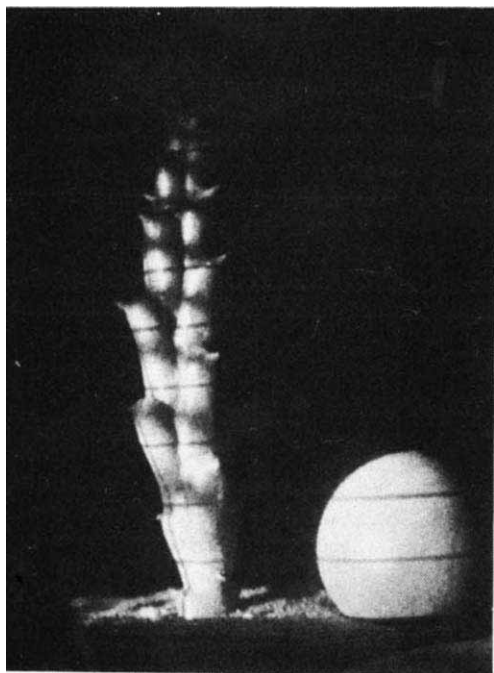


Fig. 2 Holographic interferogram of *S. variegata* Linn showing horizontal bands indicating motion of plant between exposures. Photographed through the hologram.

of a point on the object can then be calculated by evaluating the vector expression

$$N = \frac{1}{\lambda} (\mathbf{k}_1 - \mathbf{k}_2) \cdot \mathbf{d} \quad (1)$$

where N is the number of fringes counted from a stable reference point at the base of the plant to the point of interest, $\mathbf{k}_1$ and $\mathbf{k}_2$ are unit vectors in the source and observation directions respectively, $\mathbf{d}$ is the displacement vector, and λ is the wavelength of the radiation used to make the hologram.

The experimental apparatus used in the present study is illustrated in Fig. 1. The room temperature was controlled, and precautions were taken to eliminate air currents and extraneous light. *Stapelia variegata* Linn was used because it is easy to maintain in the laboratory, and its size and smooth contour result in easily discernable fringes.

To capture transient plant responses, a sequence of holographic interferograms was taken using an automatic camera loaded with a strip of Kodak SO243 35-mm holographic film. It was necessary to allow the camera to equilibrate for 1 min between advances of the film so that the new frame would stabilise thermally and mechanically. Thus the resultant sequence of double exposure holograms represents the displacements during 4-min intervals taken every 5 min. The reconstructed image of a typical holographic interferogram is shown in Fig. 2.

In the present set of experiments, the entire side of the plant was illuminated with 0.02 mW cm^{-2} 450 nm light obtained from a monochromator. This blue light was chosen because it falls in the most active area of the phototropic action spectrum of typical plants. The procedure was to wait until the plant had reached thermal and phototropic equilibrium and then suddenly expose it to a constant phototropic stimulus. The time intervals between exposures were measured to within 0.5 s using a Gralab timer.

Two main types of results were obtained from the experimental data—plots of plant displacement normal to the main axis of the plant body against height on the plant, and plots of displacement and velocity as a function of time of a par-

ticular point on the plant. Figure 3 is a typical example of the first type of result. From this data, the precise bending of the plant in response to phototropic stimuli can be studied. Figure 4 illustrates the second major result. Displacement and velocity of a point 3.5 cm from the base of a 4.2-cm high plant are plotted over a 75-min timespan. The increase in velocity on stimulus application can be shown to correlate well to a function of the form $1 - e^{-\alpha t}$, the decay on removal of the stimulus to $e^{-\alpha t}$. The solid lines in Fig. 3 are based on this assumption with $\alpha = 0.2$.

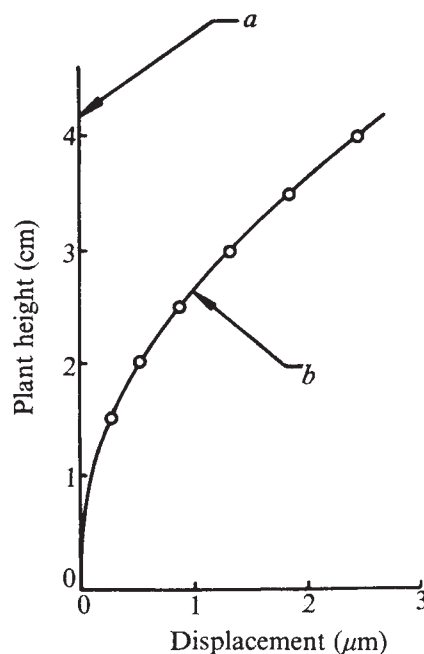


Fig. 3 Quantitative plot of the bending undergone by the plant shown in Fig. 2. The fringes represent the movement of the plant in response to 0.02 mW cm^{-2} 450 nm blue light over a 4-min time interval, after it had been exposed to 20 min of 0.02 mW cm^{-2} 450 nm blue light. *a*, plant position at time of first exposure; *b*, at time of second exposure (4 min later).

Conventional techniques for the measurement of plant motions have various limitations and shortcomings. The direct measurement of displacements by such methods as shadowgraphs and time-lapse photography has very low resolution and is therefore restricted to relatively large movements. Systems making use of mechanical displacement detectors require physical contact with the plant. Techniques using microscopes can examine only small areas on the plant at a time.

Holographic interferometry is useful for measurement of displacements in the micrometric range which can be expected to occur a few minutes to an hour after application of a stimulus. Since different mechanisms may be involved in the long term movements which have been observed with coleoptiles, holographic interferometry opens up a completely new area of investigation. Each hologram contains an image of the entire plant so that it is possible to quantify the changes with time of displacement, velocity and acceleration at any point on the plant visible from the observation point. Furthermore, the high sensitivity makes it feasible to run phototropism studies on mature plants for the first time. Holographic interferometry also provides a permanent, highly accurate record of plant movements. Due to the large amount of data contained on a single hologram, computerised analysis may be useful to obtain detailed displacement, velocity and acceleration maps over the entire plant.

This is the first time dynamic plant movement data involving such small displacements has been obtained over such a short

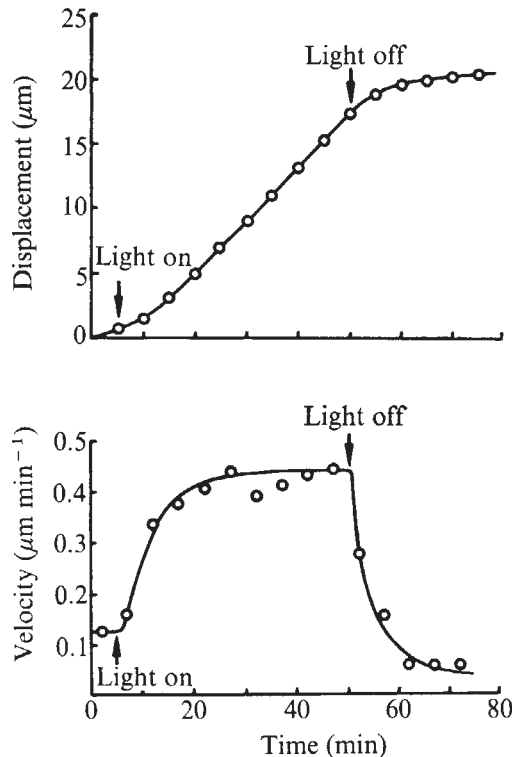


Fig. 4 Phototropic displacement undergone by a point 3.5 cm from the base of a 4.2-cm high plant in response to 0.02 mW cm^{-2} , 450 nm blue light from a monochromator. Before $t=5$ min the stapelia was undergoing a small constant motion in darkness. The blue light stimulus was applied at $t=5$ min and removed at $t=50$ min. *a*, Total tip displacement; *b*, tip velocity. The points are experimentally determined, whereas the lines represent the best fit assuming the velocity data are of the form $1 - e^{-0.2t}$ on stimulus application and $e^{-0.2t}$ on stimulus removal.

time frame. Thus, the data are of interest simply to their unique nature. In addition, the results indicate an unexpectedly rapid response to phototropic stimuli by what might be considered a sluggish plant. Perhaps the most important observation is the unexpected conformity of the velocity data to an exponential function suggesting the applicability of a second order linear differential equation to plant movement analysis. Overall, the results obtained thus far suggest that the application of holographic interferometry to plant movement analysis will make possible more complete understanding of the mechanisms of plant movement.

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Skeletal regeneration in a Red Sea scleractinian coral population

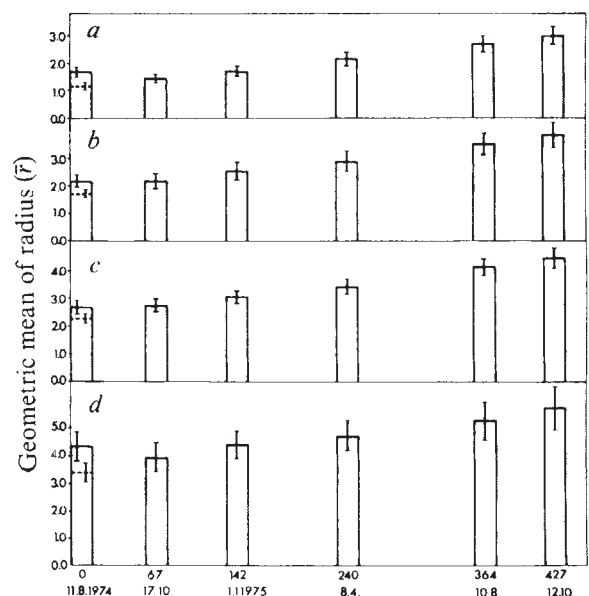
CORALS may be harmed by their natural enemies or by environmental conditions. The ability of scleractinian corals to regenerate damaged parts has been documented qualitatively by many investigators¹⁻¹⁰, but detailed quantitative data on the rate of regeneration have not been reported. Most reports dealing with the destruction of coral reefs have emphasised the long time required for recovery¹¹. I have found, however, that

the rate of skeletal regeneration in a population of the branched coral *Stylophora pistillata* (Esper) is surprisingly fast. During the first 2 months of regeneration, damaged colonies grew twice as fast as intact control colonies. Within the same colony, damaged branches grew faster than intact branches, which resulted in a tendency to regain symmetry lost through breakage. Larger colonies showed a better capacity to resist damage than smaller colonies.

Stylophora pistillata is one of the most important scleractinian corals in the Gulf of Eilat¹² because of its abundance and major contribution to the reef framework¹³. Seventy colonies of *S. pistillata*, growing in shallow water (3-4 m deep) at Eilat, were damaged by breaking some of their branches with a diver's knife. All damaged colonies were numbered by means of plastic tags and photographed before and after breakage, as well as during recovery. The growth rate of approximately 200 intact colonies studied previously in the same area¹⁴ served as a control for comparative analysis. The study lasted from August 11 1974 to October 12 1975. The length, width and height of each colony were measured under water every 2-3 months. Length is defined as the distance across a coral between the tips of branches which are farthest apart; width is a measure perpendicular to the length axis; height is orthogonal to the width and length axes. Since the shape of *S. pistillata* approximates a sphere, the colonies were divided into size groups, according to the geometric mean of their radius, $\bar{r}$ (Fig. 1).

The initial response of the coral to breakage was to cover the exposed areas rapidly with living tissue to prevent settlement of fouling organisms. Fishelson⁸ found that after a catastrophic low tide that caused partial desiccation or death of many colonies of Red Sea scleractinian corals, regeneration in

Fig. 1 Skeletal growth rate in damaged colonies of *S. pistillata*. The corals were divided into size groups according to their geometric mean radius ($\bar{r}$) after breakage. ($\bar{r} = (l \times w \times h)^{1/3}/2$ where l is length, w is width, and h is height (cm).) The $\bar{r}$ of each size group is given (in cm) by the height of the bars. The vertical lines in the middle of the bars represent the standard deviations. The horizontal broken line in the left bar of each size group represent $\bar{r}$ after breakage. The percentage decrease in $\bar{r}$ after breakage for each size group was 29.08, 19.46, 14.20 and 21.74 in order of smallest to largest size group. The estimated number of days of growth ($\pm$ s.d.) required to regain original $\bar{r}$ were 122 ± 17 , 67 ± 7 , 51 ± 5 and 116 ± 15 in order of smallest to largest size group. The expected number of days of growth required to regain original $\bar{r}$ after 25% breakage are 104 ± 14 , 86 ± 10 , 90 ± 9 and 133 ± 17 in order of smallest to largest size category. *N*, number of colonies measured. *a*, $N = 11$, $\bar{r} = 1.00-1.50$; *b*, $N = 14$, $\bar{r} = 1.51-2.00$; *c*, $N = 16$, $\bar{r} = 2.01-3.00$; *d*, $N = 10$, $\bar{r} = 3.01-4.00$.



Time of growth (d) and dates of measurement

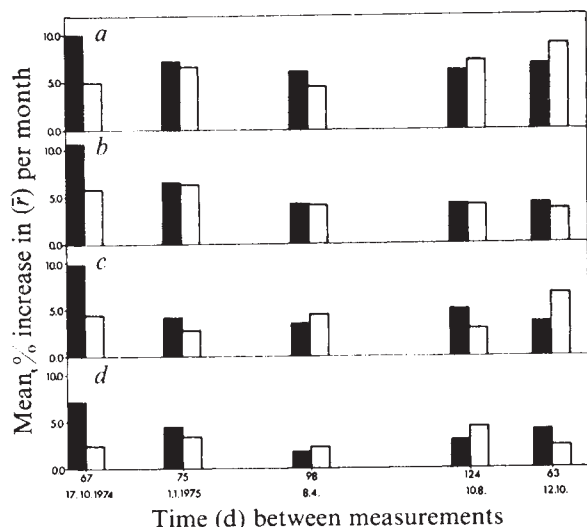


Fig. 2 Comparison between the mean percentage increase in geometric mean of radius ($\bar{r}$) per month ($\Delta\bar{r}$ %/month) of damaged corals (black bars) and intact control corals, belonging to the same size interval (blank bars). Average values of $\bar{r}$, in the different dates of measurements are given in Fig. 1.

$$\Delta\bar{r} \text{ \% / month} = \frac{30}{\Delta t} \frac{(\bar{r}_t - \bar{r}_{t-1}) \times 100}{\bar{r}_{t-1}}$$

where Δt is time (d) between measurements.

N_1 , Number of colonies measured in regenerating groups; N_2 , number of colonies measured in intact control groups. $a, \bar{r} = 1.00-1.50, N_1 = 11, N_2 = 23$; $b, \bar{r} = 1.51-2.00, N_1 = 14, N_2 = 19$; $c, \bar{r} = 2.01-3.00, N_1 = 16, N_2 = 17$; $d, \bar{r} = 3.01-4.00, N_1 = 10, N_2 = 9$.

branched colonies started with reorganisation and reactivation of the peripheral soft tissue along the line of destruction. The regenerated tissue grew over fouling algae, which had settled on the dead coral skeleton, resulting in a "sandwich structure", in which the old skeleton and newly deposited skeleton engulfed the algae. According to Connell¹⁵, if tissue damage on a coral surface is sufficiently large, recovery may not be rapid enough to exclude colonisation by fouling organisms, that may ultimately kill the coral. Fifty-one out of seventy damaged colonies survived throughout the study (Fig. 1). Most of the smallest colonies ($\bar{r} < 1.00$ cm), however, were covered by filamentous algae within a few days of being broken, and shortly afterwards they were dead. This confirms Connell's¹⁵ conclusions that larger colonies have a better capacity to resist invasion and damage than smaller ones of the same species.

Figure 1 presents the extent of skeletal damage caused to each size category and the rate of regeneration and growth. The percentage decrease in $\bar{r}$ of different size groups varied from 14% to 29%. Assuming linear growth between the periods when measurements were taken, and taking into account the different standard deviations of $\bar{r}$, within each size category (Fig. 1), I estimated the number of days of growth required by the damaged corals to attain their size before breakage (see legend to Fig. 1). To compare the different size groups with respect to their capacity for skeletal regeneration, the expected average number of days of growth to regain original size after 25% breakage was calculated for each group (see legend to Fig. 1). The largest size group ($\bar{r} = 3.01-4.00$ cm) required a significantly longer time to attain its original size than did any of the other groups (t tests, $P < 0.05$), which strengthens the assertion that this species grows more slowly as it ages¹⁴.

Figure 2 compares the mean percentage increases in $\bar{r}$ per month ($\Delta\bar{r}$ %/month) in the damaged corals and the intact control groups. During the first 2 months of regeneration, all damaged corals grew twice as fast as the intact control corals. After the damaged colonies had regained their original size, no

significant differences were observed between the average growth rate of damaged and intact colonies (t tests, $P > 0.05$). In general, the growth of both regenerating and intact colonies slowed down during the winter (December to April).

Ninety per cent of the population exhibited accelerated growth at the damaged portions of the colony (compared with intact branches) as well as a tendency to regain symmetry lost by breakage. Similar observations have been reported before³⁻⁶. This phenomenon suggests some integrated response between the parts of a single colony¹⁵: individual polyps act synergistically to differentiate tissue and create edge zones of proliferation¹⁶, and locally to accelerate skeletogenesis. The idea of integrated response is strengthened by evidence of transfer of energy-rich organic products between adjacent parts of a colony¹⁷. Thus, Pearse and Muscatine¹⁷ suggested that translocated products of zooxanthellae may enhance calcification rates in corals, by serving either as specific substrates in the organic matrix or as general energy sources.

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Persistence in continuous light of a circadian rhythm in the mosquito *Culex pipiens fatigans* Wied.

BRADY¹ has discussed Truman's² hypothesis that circadian clocks can be divided into two types: type 1 in which light seems to act directly on the oscillator, stopping it in constant light (LL); and type 2, in which the photoreceptor and oscillator are separate and the rhythm is free-running in LL. Typically, in insects, developmental events such as eclosion seem to be controlled by the first type, and daily behavioural activities by the second². In Diptera, however, behavioural rhythms seem to be rapidly damped out by LL¹; for example in the mosquito *Anopheles gambiae*, the 'clock' controlling flight activity seems to stop when the light phase is prolonged, and to restart at the next onset of darkness³, thus showing type 1 characteristics. Previously, the only known exception among the Diptera was *Aedes aegypti*, a light-active mosquito, in which the flight activity rhythm persists, rather weakly, in LL⁴. Here I show that circadian flight activity in the mosquito *Culex pipiens fatigans* Wied. persists in LL, although in other respects

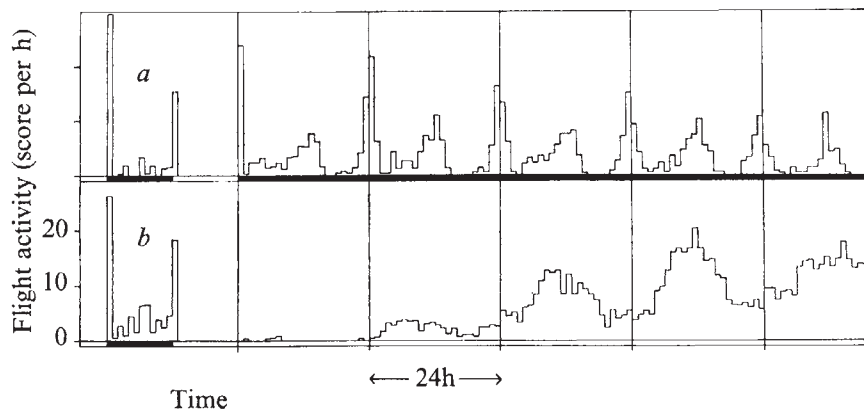


Fig. 1 Mean flight activity of individual female *C.p. fatigans*: effect of a change *a*, to DD ($n = 32$), *b*, to LL ($n = 46$).

the rhythm is very similar to that in *A. gambiae*. As in *A. gambiae*³ the period of the first cycle in constant darkness (DD) is distorted by the previous light period, but, in contrast, the magnitude of the effect depends on a rhythm that evidently persists in LL.

The culture of *C.p. fatigans* originated in the Gambia, West Africa; they were reared in alternating 12 h light: 12 h dark (LD 12:12) at 27 °C. The flight activity of individual females was monitored acoustically^{5,6} (males gave almost identical results). The light intensity used in all experiments was 50 lx.

In LD 12:12, the pattern of flight activity in *C.p. fatigans* was very similar to that in *A. gambiae*⁷ with peaks following light-off and light-on, and moderate activity during the dark phase. Cyclical activity continued in DD and, in contrast to *A. gambiae*, also in LL (Fig. 1). In DD the cycle was more strongly bimodal than in *A. gambiae*, with a secondary peak about 13 h after the main one. In LL, the cycle was unimodal with a period of about 26 h. Although activity was initially depressed in LL, it is possible to calculate that the first peak should have occurred about 18 h after light-on or 6 h after the normal time of light-off, when a small amount of activity did in fact occur (Fig. 1*b*, day 2).

When the change to DD was made after prolonging the final light phase, the rhythm was reset so that the first peak in DD always came in the first hour after light-off; shortening the final light period to 6 h did not reset the rhythm. In *A. gambiae*, light seems to have a delayed effect on the rhythm, causing a small but progressive increase in the period of the first cycle in DD, as the final light phase is prolonged³; but in *C.p. fatigans*, the effect seems to be much greater and the period of the first cycle in DD varied cyclically as the length of the final light phase was increased (Fig. 2). The period was greatest when light-off occurred about 6 h after a peak (or predicted peak) of activity in LL, and least when light-off occurred just before a peak. The response, therefore, seems to be determined by the phase of the rhythm in LL. In contrast, the lack of phase-

determined responses in *A. gambiae* implies that there is no underlying undetected driving oscillation in LL.

For *C.p. fatigans* the period of subsequent cycles in DD was approximately 24 h in all cases, irrespective of the length of the previous light phase. Although the first peak in DD followed immediately after light-off, the phase of the free-running cycle was actually determined by the timing of the second peak, which depended both on the time of light-off and on the period of the first cycle.

Thus the mosquito flight-activity 'clock' seems to behave like one of Truman's type 2 clocks in *C.p. fatigans* and possibly also in the day-active *Ae. aegypti*, but like a type 1 clock in *A. gambiae* whose behaviour resembles that of the classical *Drosophila* eclosion clock⁸. Nevertheless, in *A. gambiae*, there is strong evidence⁹ for the separation of this 'activity' clock from the 'developmental' clock controlling the time for pupation, whereas in *Drosophila*¹⁰ the same clock seems to 'gate' both eclosion and adult behaviour. It seems unlikely that flight activity is controlled by fundamentally different types of clock in two species of mosquito with such similar basic patterns of activity. The differences between *C.p. fatigans* and *A. gambiae* could simply be due to different sensitivity thresholds, the oscillation persisting in LL (50 lx) in *C.p. fatigans* but not in *A. gambiae*; related evidence³ suggests that in the latter it is damped out by 2 lx but not by 0.2 lx, although it can be entrained to a LD 12:12 regime at this intensity.

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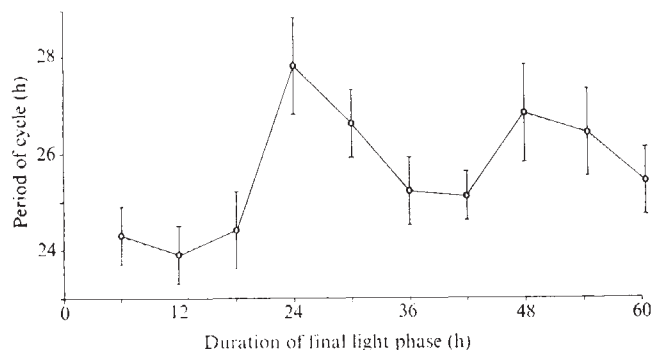
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Fig. 2 Effect on the period of the first cycle in DD of changing the duration of the final light phase.



Induction of megakaryocyte colonies with platelet formation *in vitro*

COLONY culture methods utilising semi-solid media which facilitate extensive cellular differentiation *in vitro* have proved invaluable for the quantitative investigation of granulocytic and erythrocytic progenitors and the effector substances to which they respond^{1,2}. Colonies of megakary-

ocytes have been produced from suspensions of mouse bone marrow or spleen cells in agar containing lymphocyte-conditioned medium³. But these megakaryocytes did not reach the stage of maturation at which significant platelet production could be demonstrated. We now report the development of megakaryocyte colonies with extensive platelet production in plasma cultures seeded with mouse bone marrow cells and containing either sheep or human erythropoietin in the medium. The proportional relation observed between the number of colonies produced and the number of bone marrow cells plated also provides the basis of a quantitative assay method for megakaryocyte progenitors in murine haemopoietic tissues.

Suspensions of mouse bone marrow cells were prepared from 8-week-old C57BL/6Ut mice and seeded at 1×10^5 cells in 0.5-ml volume plasma cultures prepared as described for the growth of erythropoietic bursts², except that beef embryo extract was replaced by an equal volume of medium NCTC 109 containing CaCl_2 to promote clot formation (final concentration of CaCl_2 , 0.013 mg per 0.5 ml culture). Cultures were examined daily. A few megakaryocytes were seen on day 4 and by day 5 colonies were present. These increased in number and size

up to day 7. The colonies consisted of two to 40 cells and appeared either as clusters of megakaryocytes or as megakaryocytes intermingled with the cells of an erythropoietic burst.

When graded doses of sheep or human erythropoietin were added to cultures of 1×10^5 bone marrow cells, the numbers of both megakaryocyte colonies and of erythropoietic bursts at 7 d were found to be related to the concentration of erythropoietin added (Fig. 1). At an erythropoietin concentration of 1.5 U per 0.5 ml culture, the number of megakaryocyte colonies was proportional to the number of bone marrow cells plated (Fig. 2); about 1/4,000 bone marrow cells appeared to be the progenitor of a megakaryocyte colony.

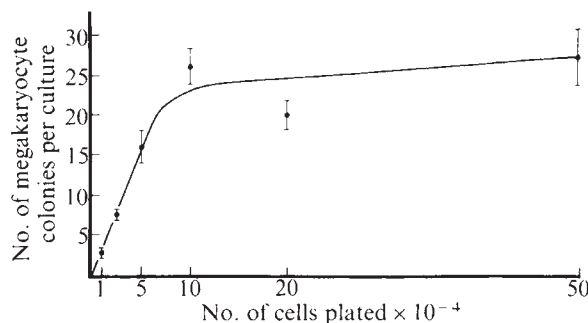
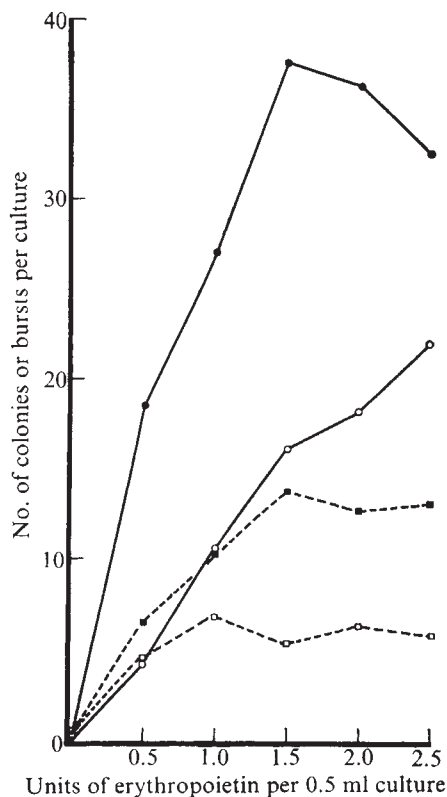


Fig. 2 Relationship between the number of mouse bone marrow cells plated and the number of megakaryocyte colonies produced after 7 d in plasma cultures containing 1.5 U of sheep erythropoietin per 0.5 ml of culture.

Fig. 1 Effect of variation in erythropoietin concentration on the number of megakaryocyte colonies and erythropoietic bursts produced in 0.5-ml plasma cultures seeded with 1×10^5 C57BL/6Ut bone marrow cells. Two preparations of erythropoietin were used: erythropoietin step III Lot. No. 3005 (Connaught Medical Research Laboratories, Willowdale, Ontario) prepared from anaemic sheep plasma (2.1 U per mg protein) and human urinary erythropoietin (pool H-11-TaLSL, 74.3 U per mg protein). Human erythropoietin was collected and concentrated by the Department of Physiology, University of the North-east, Corrientes, Argentina, further processed and assayed at the Hematology Research Laboratories, Children's Hospital of Los Angeles. ●, No. of erythropoietic bursts produced in cultures containing sheep plasma erythropoietin; ○, no. of erythropoietic bursts produced in cultures containing human urinary erythropoietin; ■, no. of megakaryocyte colonies produced in cultures containing sheep plasma erythropoietin; □, no. of megakaryocyte colonies produced in cultures containing human urinary erythropoietin.



In preparations for light microscopy megakaryocytes were identified in the cultures by their morphology. They measured 20 to 100 μm wide and contained a single nucleus consisting of 2–16 lobes. The larger cells contained the more lobulated nuclei, presumably reflecting the higher degree of ploidy in these cells. By day 6 small rounded bodies 3–6 μm in diameter were seen at the periphery of many of these large cells. Because acetylcholinesterase has been used as a cytochemical marker for megakaryocytes and platelets, cells in some cultures were stained for this enzyme. Plasma cultures were fixed in 5% glutaraldehyde and treated by the method of Karnovsky and Roots⁴. The cells in the colonies and the small bodies at the periphery of the larger cells stained an intense brownish colour, indicating a high concentration of the marker enzyme.

Serial sections prepared for electron microscopy from 'pure' megakaryocyte colonies fixed and stained *in situ* showed large cells with multilobed nuclei, surface connecting tubules and demarcation channels in their cytoplasm, all characteristic of megakaryocytes. Small rounded bodies were present at the periphery of many of these cells, measured 5 μm or less in diameter and appeared either completely separate from the megakaryocyte cytoplasm or attached to the megakaryocyte along a partly formed demarcation channel in the cytoplasm. The fine structure of these, containing α granules, dense bodies, dense tubules and surface connecting tubules, was characteristic of platelets (Fig. 3).

In this study we have shown that megakaryocyte colonies can be grown in plasma cultures from their progenitor cells in bone marrow, and that maturation of these cells in the culture conditions described results in the production of platelets. In these experiments the effector substance responsible for inducing the production of megakaryocyte colonies was probably erythropoietin. Consistent with this interpretation are reports that certain pathological conditions in which erythropoietin levels are high are associated

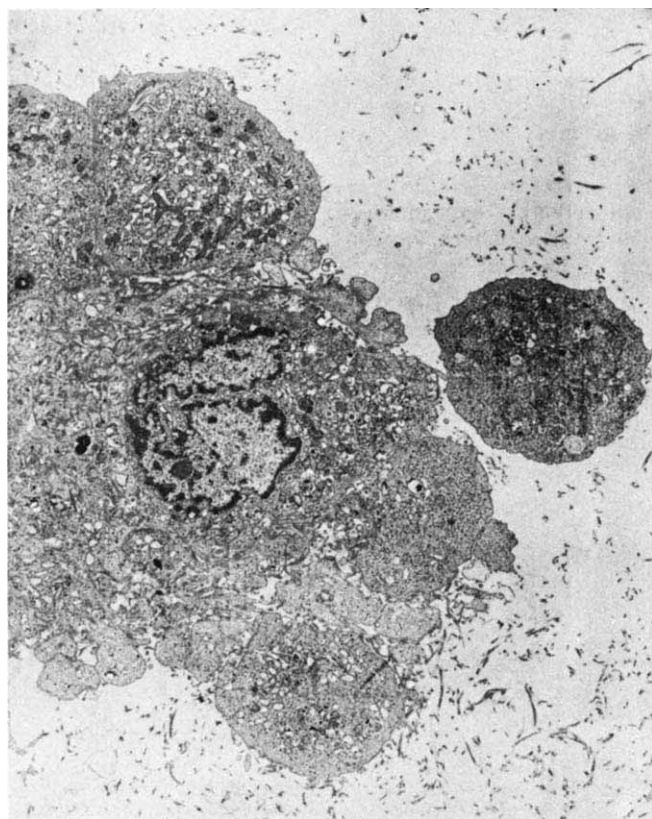


Fig. 3 Electron micrograph of a megakaryocyte from a colony that developed in plasma culture containing sheep erythropoietin, 8 d after it was seeded with C57BL/6Ut bone marrow cells. The culture was fixed in 3% glutaraldehyde, postfixed in osmic acid, flat-embedded in Epon, sectioned and examined in a Philips 300 electron microscope. Surface connecting tubules and demarcation channels are visible. The platelet at right was shown in serial sections to be separate from the cytoplasm of the megakaryocyte.

with thrombocytosis, and that correction of the primary pathological condition brings platelet levels to normal¹. It is possible that a contaminant in the erythropoietin preparations was responsible for the effect, but we believe that this is unlikely. The megakaryocyte colony-stimulating factor would then have to be present as a contaminant both in the erythropoietin prepared from the serum of anaemic sheep, and in the erythropoietin prepared from the urine of anaemic patients. Furthermore, the concentration of the contaminant would have to be approximately the same relative to erythropoietin in both preparations (Fig. 2), although the erythropoietin concentrations of the two are vastly different (sheep erythropoietin, 2.1 U per mg protein; human erythropoietin, 74.3 U per mg protein).

It is interesting that Metcalf *et al.*³ found that in cultures containing activated lymphocyte-conditioned medium, megakaryocyte colonies and granulocyte-macrophage colonies were produced, whereas in ours containing erythropoietin, megakaryocyte colonies and erythropoietic bursts were produced. The association of megakaryocyte colonies with granulocytic colonies on one hand and with erythropoietic bursts on the other raises the question as to whether the heterogeneity responsible for this phenomenon is at the level of the effector substance or of the responding progenitor cells.

The plasma culture system has already provided reliable assay systems *in vitro* for progenitors of erythrocytes and granulocytes; it should prove equally valuable for titrating megakaryocyte progenitors and for studying the mechanisms regulating thrombopoiesis.

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Susceptibility of Fanconi's anaemia fibroblasts to chromosome damage by carcinogens

INDIVIDUALS with certain genetic syndromes associated with chromosome damage show markedly increased incidence of cancer¹. In one such syndrome, Fanconi's anaemia (FA), chromosomal breakage and rearrangement has been found in lymphocytes and fibroblasts^{2,3} years before the development of malignant tumours. There is some evidence that cells from FA patients are especially sensitive to oncogenic agents. FA fibroblasts are abnormally susceptible to SV40 transformation⁴, and lymphocytes from patients show increased chromosome aberrations after exposure to ionising radiation⁵, or to alkylating agents^{6,7}. In these reports, however, evaluation was based on cells which entered metaphase during brief and possibly toxic exposures to chemicals. We report here experiments in which FA fibroblasts were exposed to a direct-acting mutagen or carcinogen for a period of 6 d, ensuring chronic exposure of the cells during one or more cell cycles, until increased cell density inhibited further cell division. After subculture we could then assay chromosome damage in cells capable of entering a new cycle of cell division after removal from the chemical. We found that viable FA fibroblasts showed increases in chromosome aberrations after exposure to the mutagen or carcinogen at concentrations that had no effect in other cell strains tested. Since fibroblasts can be maintained in serial passage, their use in this protocol makes it possible to examine residual and possibly lasting effects of the treatment.

Human skin fibroblasts between passage 6 and 14 were used for all experiments. Fibroblasts from FA patients were originally obtained from Dr M. Swift (University of North Carolina at Chapel Hill), xeroderma pigmentosum (XP) fibroblasts were from the American Type Culture Collection (cell strain CRL 1158), and normal and trisomy 18 fibroblasts were derived from skin biopsies in this laboratory. Freshly distilled diepoxybutane (DEB) and ethylmethanesulphonate (EMS) were obtained through the courtesy of B. M. Goldschmidt (New York University Medical Center). Both are alkylating agents which require no metabolic activation *in vitro*. Mutagenic and carcinogenic activity have been demonstrated for DEB⁸⁻¹¹; EMS is a well known mutagen which has been shown to be a weak carcinogen¹².

Preliminary studies were done to determine the toxicity of each compound. Cells were plated in 25-cm² flasks at a concentration of 3×10^5 cells/flask, and treatment with the chemical was begun 24 h after plating. The final concentrations of DEB in the culture medium were 1.0 $\mu\text{g ml}^{-1}$, 0.1 $\mu\text{g ml}^{-1}$ and 0.01 $\mu\text{g ml}^{-1}$. The half life of DEB in aqueous solution is approximately 4 d. Four days after plating, cultures were washed with PBS and refed, and fresh carcinogen was added at the same concentration. EMS at concentrations of 10^{-4} M, 10^{-5} M and

10^{-6} M was added to cultures daily because of its instability in solution. In each case, cells received a chronic exposure to chemical over a period of 6 d. Cell counts and dye exclusion studies using Trypan blue were done in triplicate 4 and 7 d after initial plating. That concentration of chemical which had least effect on growth rate and viability of FA cells during the 6-d exposure period was considered to be non-toxic, and was selected for use in chromosome studies. The highest concentration of DEB found to be non-toxic to FA cells was $0.01 \mu\text{g}$ per ml medium (1.25×10^{-7} M). Figure 1 shows the growth rate of treated and untreated FA and XP cells at this concentration of DEB. For EMS the non-toxic concentration was 10^{-6} M. Higher concentrations of these compounds caused a significant reduction in growth rates of cultures, altered cellular morphology, and increased cell death.

Non-toxic concentrations of the chemicals were selected for chromosome studies primarily to study the effects of low doses of carcinogens, and to maintain the largest possible dividing pool of cells for these studies. Cells in metaphase were collected 48 h after replating and growth in carcinogen-free medium since we were interested in the residual effects of chemical exposure on the surviving and dividing population of cells. The treatment schedule used in the chromosome studies was the same as that used to determine the carcinogen toxicity.

The results of treatment with the non-toxic concentration of DEB are shown in Table 1. There was a significant increase in chromosome breakage in two strains of FA cells treated with DEB when compared with the untreated FA cells ($P < 0.001$). Qualitatively, the breakage caused by the carcinogen was similar to the chromosome aberrations which occur spontaneously in these cells. Chromatid breaks were the most common chromosome aberration in the FA cells, both before and after treatment with the carcinogen. Chemical treatment increased the incidence of chromosomal as well as chromatid aberrations. Furthermore, the breaks were not concentrated in a few cells; treated populations of FA-2 showed damage in 70% of mitotic cells treated. There was no increase in aberrations with DEB treatment of XP, trisomy 18, or normal cell cultures. The inability of DEB, at the non-toxic concentration, to induce any chromosome aberrations in these other genetically abnormal cells, was in sharp contrast to its effect on FA cells.

Treatment of FA cells with EMS also resulted in a significant increase ($P < 0.001$) in chromosome aberrations when compared to untreated FA cells (Table 2). Furthermore, no aberrations

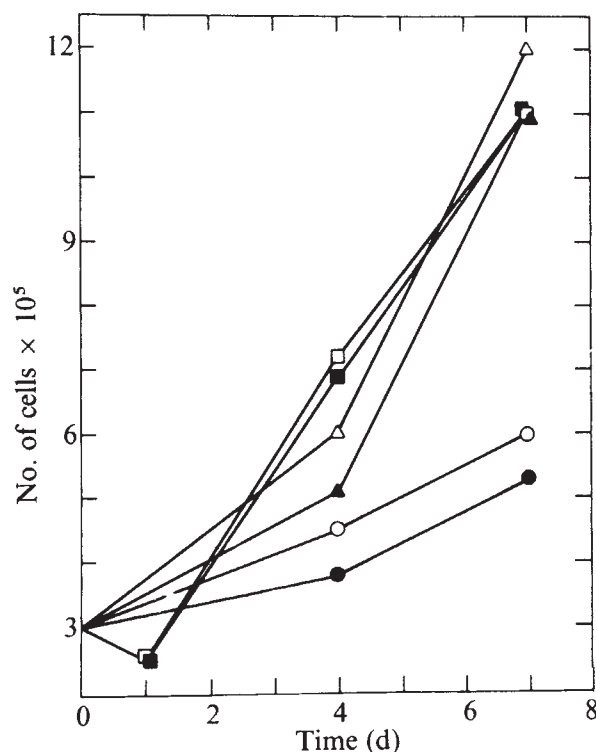


Fig. 1 Parallel cultures (open and closed symbols) showed similar increases in cell number in presence or absence of carcinogen treatment (DEB at $0.01 \mu\text{g m}^{-1}$). Normal and trisomy 18 cells grew more rapidly, growing from 3×10^5 to $18-24 \times 10^5$ cells in 7 d. These cell strains also showed no difference in growth rate between treated and untreated cells. $\circ$: FA-1, control; $\bullet$: FA-1 treated with DEB; $\square$: FA-2 control; $\blacksquare$: FA-2 treated with DEB; $\triangle$: XP, control; $\blacktriangle$: XP, treated with DEB.

were induced in normal cells at the same non-toxic concentration of EMS. When the incidence of breakage in FA cells induced by DEB was compared with that induced by EMS, the concentration of each chemical being equivalent in its cytotoxic effects, the difunctional agent DEB was found to be a significantly more potent karyolytic agent ($P < 0.01$).

Statistical significance of the increase in breakage of treated

Table 1 Chromosome breakage persisting after exposure to DEB ($0.01 \mu\text{g ml}^{-1}$) for 6 d

Cell type		Chromatid breaks	Chromosome breaks	Acentric fragments and deletions	Rearrangements*	No. of breaks per cell (50 cells per set)
NSF	Untreated	0	0	0	0	0
	Treated	0	0	0	0	0
XP	Untreated	0	0	1	1	0.06
	Treated	0	0	0	0	0
FA-1	Untreated	5	6	1	0	0.24
	Treated	16	9	7	1	0.68
FA-2	Untreated	7	2	1	0	0.20
	Treated	38	8	3	3	1.10
FA-2 (repeat)†	Untreated	16	1	1	0	0.36
	Treated	35	4	3	4	1.00
Trisomy 18	Untreated	3	1	0	0	0.08
	Treated	6	0	0	0	0.12

NSF: normal; XP: Xeroderma pigmentosum; FA: Fanconi's anaemia.

*Rearrangements included multiradials, dicentric and translocations.

†The above strain was retested 10 months later with a different batch of chemical and at a different passage level.

Table 2 Chromosome breakage persisting after exposure to 10^{-6} M EMS for 6 d

Cell type		No. of breaks/No. of cells	No breaks	1 break	2 breaks	3 breaks	Total no. of cells with breaks
NSF	Untreated	0/100	100	0	0	0	0
	Treated	6/100	95	4	1	0	5
FA-2	Untreated	28/100	74	24	2	0	26
	Treated	61/100	53	35	10	2	47

versus untreated cells in each population was determined by the use of a *t* test with ∞ d.f. and the Poisson standard error. The numbers of cells with one break, two breaks, and so on, fit the expected distribution for Poisson events in both DEB and EMS treated FA cells.

These experiments separate the chromosome-damaging effects of the chemical agents from their toxic effects. We assume that selection during the chronic exposure to alkylating agents was minimal since the growth and viability of cells was not reduced. Furthermore, the same non-toxic concentrations of chemicals had no effect on either viability or rate of chromosome aberrations in the other cell strains tested. The finding of increased chromosomal aberrations in FA fibroblasts is therefore further evidence that the defect in this disease results in increased chromosome fragility in these cancer-prone individuals.

Both xeroderma pigmentosum and trisomic patients are also reported to show increased susceptibility to malignancy. Experimentally a reduction in repair synthesis has been demonstrated in XP cells after exposure to certain chemical carcinogens¹³ and trisomy 18 fibroblasts have shown an increased susceptibility to SV40 transformation¹⁴. Although both are genetically abnormal, neither of these cell types shows the levels of chromosome aberrations found spontaneously in FA cells, which may account for the difference in chromosomal effects of the carcinogen. A biochemical basis for chromosome fragility in FA has not yet been delineated, but there is some evidence suggesting FA lymphocytes are defective in a step in DNA repair^{15,16}.

The persistence of chromosome aberrations in FA cells after exposure to a carcinogen suggests a mechanism for cellular evolution to malignancy. In ataxia-telangiectasia, another disease in which there is both increased chromosome breakage and increased risk of malignancy, a consistent chromosomal rearrangement of chromosome 14 was observed, which then persisted in a leukaemic clone¹⁷. Chromosome breaks themselves are unstable, and cells with single breaks would not contribute to the evolution of chromosomally abnormal cell clones. Exposure to chemical mutagens or carcinogens in concentrations too low to inhibit cell growth and viability but sufficient to increase the number of chromosome breaks in susceptible cells, would, however, greatly increase the probability of having more than one break per cell, and, therefore, the possible formation of a chromosome rearrangement. If such rearrangements are related to the formation of malignant cell clones, then these experiments provide evidence that the increased susceptibility to malignancy in FA patients is related to the increased susceptibility of FA cells to chromosome damage from mutagens or carcinogens.

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Natural tranquilliser in cephalopod eggs

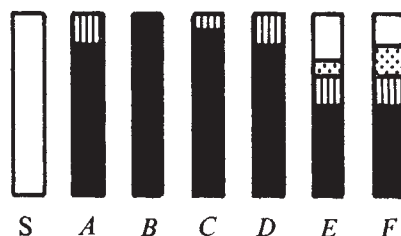
THE hatching gland of cephalopods^{1–5} is fully differentiated and may fulfil its function before the embryo has reached a stage of development sufficiently advanced for life in the open sea^{6,7}. It is not known why premature hatching occurs only in clearly artificial conditions. Investigations of the properties of the perivitelline fluid (PVF) of squid eggs have given us an important clue: this fluid acts as a tranquilliser on the young animals as they approach the end of embryonic development.

Our observations were made on eggs of the common squid *Loligo vulgaris*. The PVF of this species⁸ is a clear, slightly viscous liquid that is hypertonic to seawater. Its volume in a single egg increases from about 0.3 ml early in development to 100–130 ml towards the time of hatching. The substances that cause water uptake through the chorion are emitted by the embryo⁹; their chemical nature is unknown. Some secretory products of the epidermal glandular cells have been demonstrated by haemagglutination tests¹⁰. Although the PVF which is the natural medium of the developing embryos has a composition different from seawater, embryos develop normally in sterile seawater¹¹. The technique of collecting embryos and PVF is simple: the choria are opened with watchmaker's forceps and the contents are collected in glass receptacles¹⁰.

Animals ready to hatch, which were collected in this way, became very active when placed in seawater. They swam around by jet propulsion and remained above the bottom of the receptacle, with their chromatophores active. When they were transferred to PVF, however, active swimming ceased within about 10 s. The animals sank to the bottom, where they exhibited little or no chromatophore activity. They also made little or no response to disturbance such as knocking on the receptacle, which induced an immediate escape reaction in animals kept in seawater. When returned to seawater, quiescent animals resumed active swimming within 5 s. PVF diluted 1:1 with seawater gave an effect similar to that of PVF.

Differences in pH, in O₂ content and viscosity that exist between the PVF and seawater seem likely to be responsible for the differences observed in the behaviour of the animals^{12–14}. Preliminary experiments have shown that a lower pH, corresponding

Fig. 1 Pattern of activity compared with quiescence in hatchlings of *Loligo vulgaris* after 1 h of exposure to seawater (S); untreated PVF (A); PVF centrifuged for 1 h at 5,000 r.p.m. (B); PVF filtered through cotton (C); PVF filtered through glass fibre wool (D); PVF Millipore-filtered, 0.45- μ pore diameter (E); PVF Millipore-filtered and then heated at 100 °C for 15 min (F). Millipore-filtration of PVF must be preceded by the treatment indicated in B, C and D to avoid clogging the filter. The behaviour of the embryos in pretreated PVF was similar to that observed in A. Each test vessel (Petri dish, 6 cm diameter) contained 12 ml of fluid. There were 12 animals per vessel. The clear difference between S and E and F shows the presence of tranquillising agent even after filtration and heating. Open shading, active swimming; dots, hovering (compensation of sinking); vertical hatching, lying on bottom, ventral side up, with occasional jets produced; solid shading, continually quiescent.



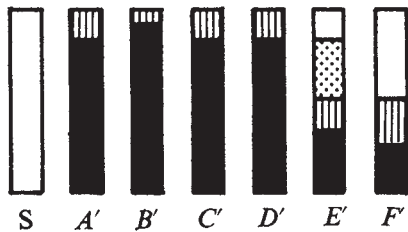


Fig. 2 Pattern of activity in hatchlings of *Loligo vulgaris* after 1 h of exposure to seawater (S) and diluted PVF at a concentration of 6.6% in seawater. A' is the dilution product of A and so on. Conditions were otherwise identical to those for Fig. 1. Shading as for Fig. 1.

to that of PVF (pH 7.5), does not influence swimming activity in seawater. In O_2 -enriched PVF animals were activated only momentarily. Thus viscosity could be a mechanical tranquilliser.

Centrifuging and/or filtering through cotton and glass fibre wool did not alter the effect of PVF (Fig. 1 B, C and D). Only after the highly viscous substances secreted by the epidermal glandular cells¹⁰ had been removed by Millipore filtration (Fig. 1E), did some animals become active. Yet some remained inactive. Thus there must be an actual tranquillising agent that is not eliminated by filtration; the inactive animals were presumably more sensitive to this component than the active animals. The heat stability of this remaining agent (Fig. 1F) suggests that it has low molecular weight. Its mild effect was complemented by the viscosity-causing substances in the untreated fluid (Fig. 1A), which seemed to impede motion mechanically. Embryos were immobilised in seawater to which methyl cellulose (4 mg ml^{-1}) had been added. In contrast to embryos tranquillised by PVF, these embryos had expanded chromatophores. The tranquillising effect of untreated fluid was observed at concentrations as low as 1.7% (Fig. 3A'–D'), whereas Millipore-filtered fluid containing only the supposed specific tranquilliser had an effect only at higher concentrations (6.6%, Fig. 2E' and F').

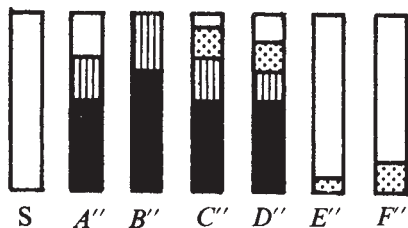


Fig. 3 Pattern of activity in hatchlings of *Loligo vulgaris* after 1 h of exposure to seawater (S) and diluted PVF at a concentration of 1.7% in seawater (compare Fig. 2). Shading as for Fig. 1.

Untreated PVF of *Loligo vulgaris* also had a strong effect on small crustaceans (mysids, prawns and copepods), small fish (about 2 cm long) and young cephalopods of other species (*Sepioida* spp.). They were immobilised within a few seconds. In our experiments some died and others resumed their normal activities within 5–10 h in seawater even after exposure to PVF for 3–4 h. No effect was observed on the ciliary activity of ciliates and on the flagellar movement in spermatozooids of sea urchins (*Paracentrotus lividus*); these spermatozooids agglutinated however^{10,15}.

For the cephalopod embryo, the protective effect of a tranquillising mechanism is twofold: it prevents premature hatching which would mean the hatching of less viable young, and the waste of energy by muscular activity. The result is optimum conditions for the survival of well developed hatchlings and thus is part of what can be called the reproductive strategy.

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Action of papaverine and ionophore A23187 on neurulation

MICROFILAMENTS, which are thought to be activated by Ca^{2+} , are functionally related to the apical constriction of neural plate cells during neural tube morphogenesis. Papaverine interferes with Ca^{2+} flux and inhibits neurulation, disrupting microfilament structure, while ionophore A23187 which induces Ca^{2+} flux counteracts this inhibition restoring microfilaments and promoting formation of neural ridges and folds in 10 min. We conclude that microfilament-generated morphogenetic movement may be regulated, in part, by Ca^{2+} availability.

Cytokinesis¹, epithelial morphogenesis² and egg cortex contractility³ all require calcium ions and the presence of microfilaments at regions of localised contractions. Cytochalasin B alters the morphology of microfilaments and inactivates their apparent capacity to produce the changes in shape cited above⁴. The relationship of calcium to these activities is not completely known; cortical contractions are induced in *Xenopus laevis* eggs and embryos by calcium injections⁵, and by treatment with ionophore A23187, which promotes increased membrane permeability to divalent cations such as Ca^{2+} , while activating the cortical contractile system in the frog⁶. Papaverine, in contrast to ionophore A23187, is reported to inhibit the release of bound calcium⁸. Salivary gland morphogenesis does not proceed characteristically in papaverine, a result ascribed to interference with calcium activation of microfilaments⁷. Schroeder has proposed that microfilaments contribute to the movements of gastrulation and neurulation⁹ and has demonstrated that specific arrays of microfilaments correlate with apically constricted neural plate cells⁴. We reasoned that if the timely release of calcium were causally related to the activation of microfilament contraction in neurulation, that papaverine should likewise inhibit this process and that ionophore A23187 should reactivate it.

More than 100 early neurula embryos of the amphibian *Ambystoma maculatum* late stage 13 (Harrison) were completely decapsulated and exposed to papaverine or spring water alone for a period of 10–18 h at 20 °C. The animals were distributed among spring water solutions with drug concentrations of 25, 50, 75, 150 and 300 $\mu\text{g ml}^{-1}$. Papaverine treatment disrupts neurulation; the embryos assume a spherical shape, become flaccid, and develop none of the morphogenetic features associated with the developing anteroposterior neural axis (Fig. 1a). Depending on the concentration, the neural groove may narrow or deepen and opposite sides come into contact but in papaverine concentrations greater than 50 $\mu\text{g ml}^{-1}$, neural ridges and folds never erupt as they do in the controls (Fig. 1b).

For electron microscopic observations embryos were fixed

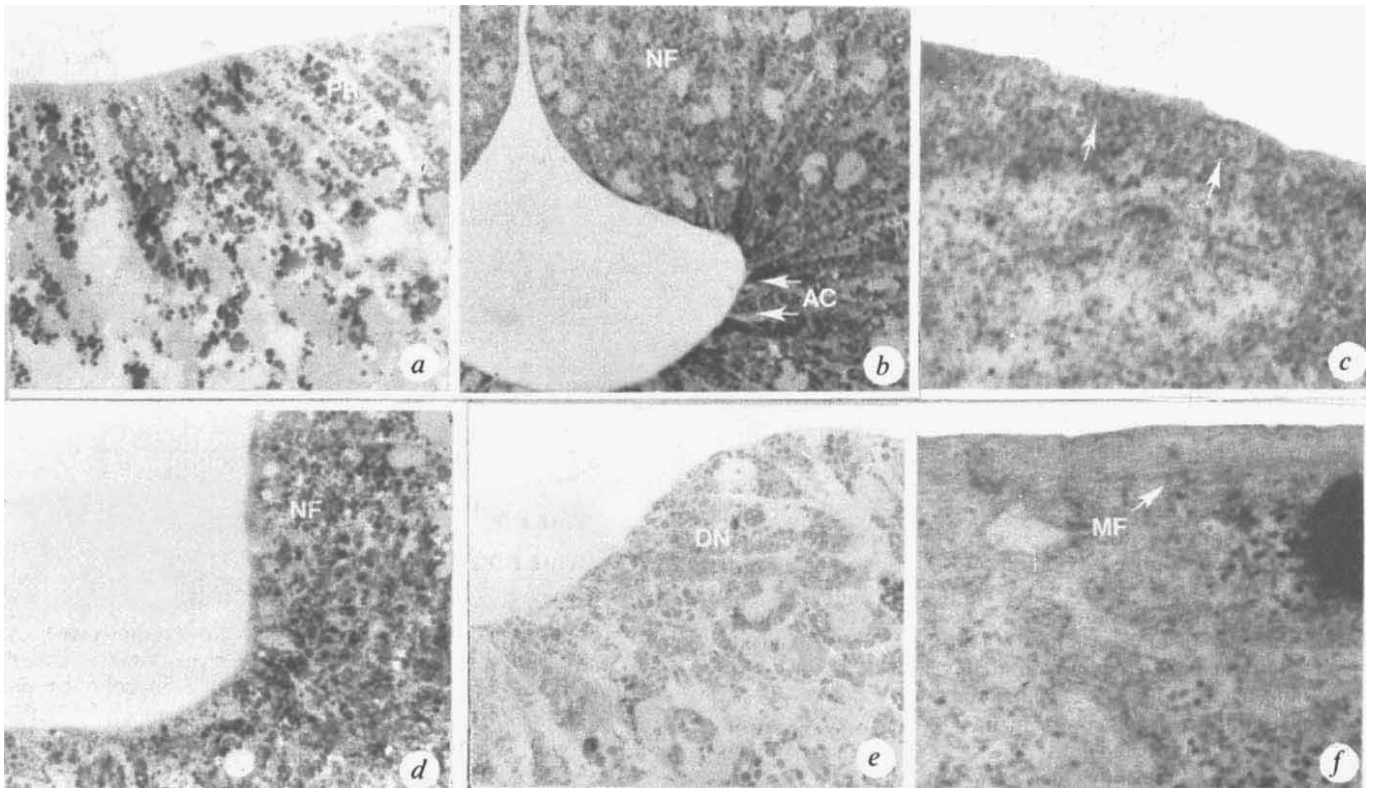


Fig. 1 *a*, Light micrograph of the neural plate of a papaverine-treated ($75 \mu\text{g ml}^{-1}$) animal. The neural ridges and folds fail to form, 163. PR, presumptive ridges. *b*, Light micrograph of a neural fold of comparable-aged control animal in spring water. Arrows (AC) indicate the position of apically constricting microfilaments of neural fold cells, 325. NF, neural fold. *c*, Electron micrograph of a neural ridge cell of a papaverine-treated ($75 \mu\text{g ml}^{-1}$) animal. Note the apical band of linear microfilaments is not present (arrows), 23,400. *d*, Light micrograph of a neural fold which developed on returning a papaverine-treated ($75 \mu\text{g ml}^{-1}$) animal to spring water for 12 h, 241. NF, neural fold. *e*, Light micrograph of a developing neural fold of a papaverine-treated ($75 \mu\text{g ml}^{-1}$) animal removed to ionophore A23187 ($25 \mu\text{g ml}^{-1}$) for 5 min, 241. DN, developing neural fold. *f*, Electron micrograph of a neural ridge cell of a papaverine-treated ($75 \mu\text{g ml}^{-1}$) animal removed to ionophore A23187 ($25 \mu\text{g ml}^{-1}$) for 5 min. Note the restoration of the apical band of microfilaments, 48,750. MF, microfilaments.

with 2.5% glutaraldehyde in Millonig's buffer and postfixed in 1.0% buffered osmium tetroxide. Specimens were embedded in Epon, mid-trunk sections were cut with a Porter-Blum MT2-B ultramicrotome and examined with a Philips EM300 operating at 60 kV. Exposure to papaverine results in distinctive ultrastructural alterations of neural ridge cells. The dense band of microfilaments beneath the apical plasma membrane in control cells is not present in either grazing or vertical sections (Fig. 1c). Pigment granules are arrayed with unusual linearity beneath the plasmalemma. The normally planar apical surface develops blunt projections, mitochondria display vacuoles and loss of cristae, and gaps appear between the usually tightly apposed cells.

If papaverine treatment does not exceed 24 h, washing the animals and returning them to spring water results in a reinitiation of neurulation, with neural ridges and folds forming (Fig. 1d) and fusing within 1 d. This reversible inhibition by papaverine and its putative interference with the requisite calcium flux of neurulation provided us with a model system with which to assess the import of Ca^{2+} in these events by counteracting papaverine's stabilisation of calcium with the cation ionophore A23187. Stock solutions were prepared with a 1 : 3 solvent mixture of *N,N*-dimethyl formamide and 95% ethanol. Working solutions of 10 and $25 \mu\text{g ml}^{-1}$ were made by dilution with spring water. Control series were run in spring water and diluted solvent solutions and showed no disruption of morphogenesis.

After washing, a 10-min exposure of papaverine-inhibited animals ($75 \mu\text{g ml}^{-1}$ to $25 \mu\text{g ml}^{-1}$ of ionophore A23187) results in eruption of the neural ridges (Fig. 1e), which then rapidly form discrete folds, not always in their normal medial orientation. More than 10 min of ionophore treat-

ment promotes cell disaggregation and the subsequent collapse of the entire embryo. Electron microscopic observations reveal that dramatic fine structural changes occur during the short time in the A23187 solution. The subplasmalemmal dense band of microfilaments is reconstituted (Fig. 1f), with the pigment granules redistributed throughout the cytoplasm. Surface projections and gaps between the cells are not seen, specialised cell junctions (that is, desmosomes) are evident, and the mitochondria appear intact with numerous cristae. Microtubules, which were also seen in papaverine-treated cells, are arrayed along the vertical cell borders and within the apical dense band.

We have recently proposed a model of neurulation based on cumulative evidence of the involvement of mechanical forces and a directional guidance system together with our reports of supracellular participation of cell surface material (CSM) in the alignment and fusion of the neural folds⁹. Cellular movements and shape changes during neurulation apparently result from selective constriction and elongation. The constrictive force has been attributed to microfilaments⁸, while elongation seems related to the presence of microtubules¹⁰⁻¹². If the activity of microfilaments during the eruption and convergence of the neural ridges and folds depends on the continued availability of endogenous calcium, deactivation by papaverine should be counteracted by the effect of calcium ionophore A23187. Our experiments confirmed this, demonstrating an ionophoric induction of the morphogenetic movements of neurulation. The precise accomplishment of complex morphogenetic events such as this depends on appropriate temporal cues which, in turn, must interrelate with the spatial location and structural differentiation of diverse cells and tissues. Our data impli-

cate calcium as an instructive agent in these activities. The experiments of Barth and Barth^{13,14} earlier suggested the involvement of ions in amphibian development, most notably Ca^{2+} during neurulation, when its incorporation was shown to be markedly raised.

During embryogenesis, microfilaments develop concomitantly with cellular motility and alterations in shape^{11,12,15}. It has been suggested that these organelles accommodate cellular constriction and movement in a manner similar to the actin and myosin filaments in the sliding hypothesis proposed for muscle cells¹⁶. By analogy with muscle cells, non-muscle cells might also require Ca^{2+} for contractile activation¹⁵. The results presented here provide support for this idea and agree with the findings of Schroeder and Strickland⁹ regarding ionophore-mediated Ca^{2+} release and its subsequent triggering of the cortical contractile system. Our electron microscopic observations indicate that papaverine disruption of normal Ca^{2+} ion mobility affects cell junctions, microfilaments and mitochondria, all of which are involved in cell movement. Microtubules, which are thought to be responsible for the directionality and structural stability of moving cells or tissues, do not seem to be affected by papaverine treatment but more subtle effects cannot be precluded by our experiments. It should be noted that *in vitro* experiments have demonstrated the relation of Ca^{2+} and microtubule metabolism; high concentrations of Ca^{2+} inhibit their polymerisation¹⁷. Microtubules do not, however, seem altered by ionophore treatment. It may be, therefore, that they are normally constituted and functioning before microfilament activation, so that the high titre of Ca^{2+} induced by the ionophore only stabilises them, and does not affect their assembly. Further work is needed on this point.

Correlations between cell movement and CSM accumulation during gastrulation and neurulation have been established^{9,18,19}. As the neural axis forms extracellular materials provide an adhesive link between the convergent folds. This CSM is negatively-charged, stains avidly with Alcian blue and may, as in related amphibian systems²⁰, be polymerised and distributed at the cell surface by interactions involving Ca^{2+} . In summary, the present study implicates Ca^{2+} as a mediator of microfilament contraction and suggests the possibility that selective release of this ion may be involved in the mechanism by which non-muscle cells regulate their movement.

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Loss of a critical neutral protease in ageing WI-38 cells

THE limited lifespan of normal human diploid cells *in vitro* seems to be related to the number of mitotic divisions the cells have undergone rather than to passage of time¹⁻³. In spite of considerable research³⁻⁷ it is still unclear what biochemical events actually mediate the cell death or more accurately the absence of growth. In other systems it has been postulated that regulation of growth may be mediated by activity at the cell membrane. Specifically it has been suggested that cellular division is controlled in part by membrane mediated cell contact, and proteases and lysosomal enzymes have been implicated in the control of mitosis and growth⁸⁻¹¹. Thus cell surface events may be either primary or secondary events mediating cell ageing. Acid hydrolases have been postulated as modifying the cell surface in sublethal autolysis, electrophoretic mobility of cells gives an indication of cell surface chemistry and neutral proteases have been implicated in modification of cell external surfaces⁹⁻¹⁵. We now demonstrate that two cell surface parameters are greatly affected by *in vitro*

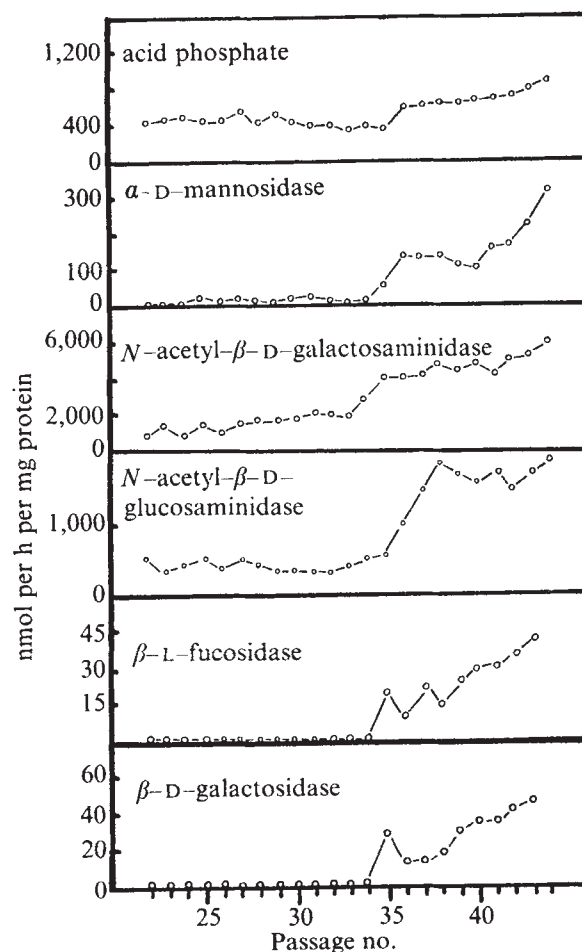


Fig. 1 Activity of six acid hydrolases as a function of passage of time in WI-38 cells. Values are means from 4 separate determinations. Glycosidase and acid phosphatase activity was determined as given previously^{12,16}, on 0.1% Triton X-100 homogenates of the cells using *p*-nitrophenyl substrates. Details of cell culture and definition of passage time are given in the text. In this and all succeeding figures the passage points are not connected because it is not clear that the activities of successive passages are truly a continuous function. It should be noted that the inability to detect activities of several lysosomal enzymes in young and middle aged cells merely reflects the relative insensitivity of the method of assay and does not preclude low levels of these enzymes being present.

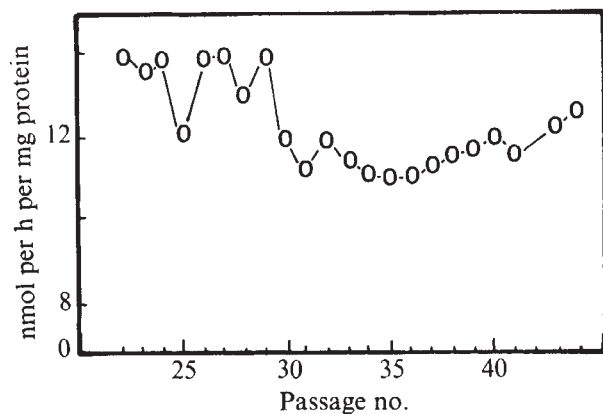


Fig. 2 Activity of cathepsin-like proteolytic activity at pH 3.4 as function of passage time in WI-38 cells. Values are means from 4 separate determinations. Proteolytic activity at pH 3.4 was determined as given previously^{17,18} on 0.1% Triton X-100 homogenate of the cells using ³H-acetyl haemoglobin as substrate. ³H-acetyl haemoglobin was prepared as described previously^{17,18}. ³H-acetic anhydride (400 Ci mol⁻¹) was reacted with the type I beef blood haemoglobin. The material was purified to a specific activity of 162 c.p.m. pmol⁻¹ based on a molecular weight of 68,000 using the counting procedures described here. The specific activity of the material was 300 Ci mol⁻¹. Routinely in the assay systems described below, 50 μ l (136 μ g ³H-acetylated haemoglobin; 2 nmol; 324,000 c.p.m.; 0.6 μ Ci) of the ³H-acetylated haemoglobin was added per assay. Routinely 100 μ l of the 0.1% Triton X-100 homogenates (between 50 and 100 μ g protein) were added to 100 μ l 1.35 M acetic acid, 0.2 M ammonium sulphate buffer (pH 3.4). As the substrate, 50 μ l of ³H-acetyl haemoglobin (2 nmol) were added. This mixture was incubated for 1 h at 37 °C in a Dubnoff metabolic shaker. The reaction was terminated by placing the assay tubes in an ice-water bath (0–4 °C) and adding 100 μ l of 2.5% haemoglobin and 50 μ l of 50% trichloroacetic acid (TCA). The precipitated material was removed by centrifugation at 5,000g for 5 min, and an aliquot of the supernatant fluid was plated on a glass fibre filter; the radioactivity was determined by counting in a liquid scintillation counter^{17,18}. Activity is expressed as nmol haemoglobin degraded per h per mg protein.

ageing: activity of a pH 7.8 protease and cell surface net charge as measured by electrophoretic mobility. It should be emphasised that the small standard deviations for the electrophoretic mobility data reported herein are a function of the large n (number of independent observations) and do not preclude population heterogeneity.

WI-38 cells at various passages were kindly provided by Dr L. Hayflick. To avoid confusion the culturing conditions used for determining passage number will be precisely described. Cells were grown, maintained, subcultured, and passage number determined exactly as given on p. 615 of ref. 2. The number of cells was divided in half every 3 d and then replated in fresh Eagle's basal medium (Gibco; G-13 powdered media) with 10% of calf serum, penicillin (500 U ml⁻¹) and streptomycin (1 g l⁻¹). Trypsin at a concentration of 0.025% was used to remove the cells from the monolayer. Thus passage number is determined herein by the "2:1 cell split method"; furthermore, all cells used for the experiments herein were grown on glass. Finally, it is important to describe the behaviour of the cells in our hands. To all outward appearances the cells grew normally until passage 35/36, when a definite decrease in the density of the cell population occurred at each harvest and subcultivation. By passage 44 the cells were so sparse it became impossible to obtain enough cells for all the biochemical procedures. Thus the cells ceased to grow at passage 44 (these cells would thus be "end phase III" cells using the nomenclature of others⁶). We did manage to carry a limited number of cells to passage 48 for one experiment but that required a great

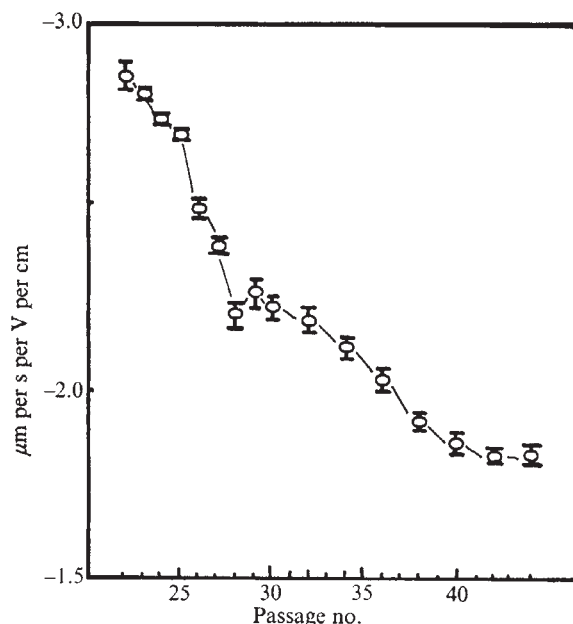
number of flasks and large quantities of medium. For all analyses (not passages) cells were washed by centrifugation at 500g for 3 min and resuspended in the above described medium without the serum before use. This procedure took 20 min.

Acid phosphatase (EC 3.2.3.2), α -D-mannosidase (EC 3.2.1.24), N -acetyl- β -D-glucosaminidase (EC 3.2.1.30), N -acetyl- β -D-galactosaminidase (EC 3.2.1.-), β -L-fucosidase (EC 3.2.1.38) and β -D-galactosidase (EC 3.2.1.23) were assayed on 0.1% Triton X-100 homogenates of the WI-38 cells as described previously using p -nitrophenyl substrates^{12,16}. Proteolytic activities at pH 3.4 and 7.8 (refs 17 and 18) were assayed on 0.1% Triton X-100 homogenates of the WI-38 cells using ³H-acetyl-haemoglobin as substrate. Electrophoretic mobility of the cells was measured as described previously^{16–20}. Protein was determined by the method of Lowry *et al.*²¹.

The data of Fig. 1 show that each of the acid hydrolases (thought to be primarily but not exclusively lysosomal) increased as the cell passage number increased. A rather marked increase occurred at passages 34–37 and the increase continued until passage 44; activity of each of the six enzymes studied per mg protein reached a maximum at passage 44. Passage 36 was the passage in which the cells began to grow less densely and this coincides with the increases in acid hydrolase activity. A sharp increase in activity occurred for β -D-mannosidase, which from passage 22 to passage 34 hovered around 0 (undetectable) activity and in β -D-galactosidase and β -L-fucosidase both of which had total 0 (undetectable levels) before passage 35. With regard to *in vitro* ageing it may be as important to determine why those enzyme levels are so low (0) before passage 35 as it is to determine why the increase occurs at late passages until passage 44. Activity levels of acid hydrolases ("lysosomal hydrolases") have been reported in general to increase on ageing *in vitro* (see for example refs 6, 7, 22, 23) and we now confirm many earlier observations.

Fig. 2 gives results of the pH 3.4 activity protease

Fig. 3 Electrophoretic mobility of WI-38 cells against passage number of the cells. Values are means $\pm$ 1 s.d. for approximately 120 independent particles at each point. Electrophoretic mobilities were determined as previously described^{18,20}. Values are for cells measured in 0.0145 M NaCl, 4.5% sorbitol, 0.6 mM NaHCO₃ (pH 7.2 $\pm$ 0.1) at 25 °C $\pm$ 0.1, as previously described^{18,20}. Ionic strength = 0.015 g ions l⁻¹. These data reflect population net negative surface charge. Because of the very high number of independent observations (> 120) the standard deviations are small.



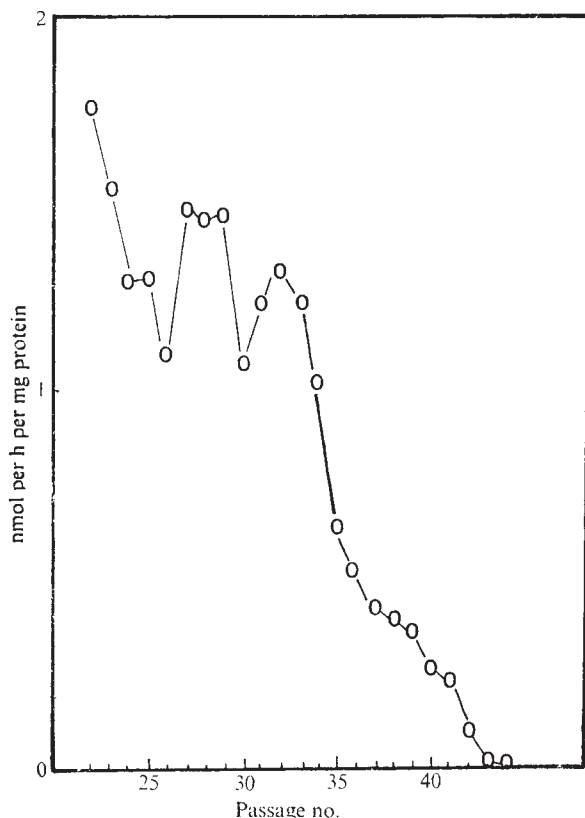


Fig. 4 Activity of trypsin-like proteolytic activity at pH 7.8 as a function of passage time in WI-38 cells. Values are means from 4 separate determinations. Proteolytic activity at pH 7.8 was measured exactly as given in Fig. 2 except that 0.1 M phosphate buffer (pH 7.8) was used in the assay in place of the pH 3.4 buffer^{15,18}.

(cathepsin-like) thought by many to be of lysosomal origin. Although high activity levels of this enzyme are present in the WI-38 cells the enzyme activity seems to only decrease slightly at passages 30-40 and increase slightly at passages 40-44.

The electrophoretic mobility data shown in Fig. 3 are unique. The data presented show that the net negative surface charge decreases from -2.82 ± 0.04 at passage 22 to -1.82 ± 0.02 at passage 44. The decrease is most pronounced at passages 25-28 and levels off at passages 42-44. On the basis of work on other mammalian cells and organelles¹⁸⁻²⁰, it is tempting to speculate that the decrease is related to a loss of external surface *N*-acetyl-neuraminic acid; such conclusions must, however, await chemical data which require large numbers of cells.

The most interesting observation is shown in Fig. 4. The pH 7.8 neutral protease activity essentially disappears at passages 43 and 44. Specifically, the activity of the enzyme goes to 0 or non-detectable activity at passage 43/44 from a passage 22 activity of 1.68 nmol per h per mg protein. To test this finding further in separate experiments passage 27 cells were grown from frozen stock (liquid N₂) and compared with cells that had grown out to passage 48. The passage 27 cells had 1.5 nmol per h per mg protein of pH 7.8 protease activity and the passage 48 cells had 0 nmol per h per mg protein of pH 7.8 protease activity (passage 27 cells had 427 nmol per h per mg protein of acid phosphatase activity and passage 48 cells 917 nmol per h per mg protein of acid phosphatase activity; see Fig. 1). This neutral protease is thus present in the rapidly dividing early passage WI-38 cells and absent from the senescent late passage WI-38 cells.

To ensure that the loss of pH 7.8 activity was not due to differential binding between phase II and phase III of

the enzyme used to remove the cells from the monolayer, the following experiments were performed. Using 0.0025, 0.025, 0.25 or 1% trypsin or removal of the cells with a rubber policeman rather than the usual 0.025% trypsin for passage 27-30 and passage 42-44 cells did not at all affect the results. That is, the passage 27-30 cells had the relatively high activity exactly as shown in Fig. 4 whereas the passage 42-44 had no measurable activity as given in Fig. 4. Thus the data shown in Fig. 4 are not an artefact of the trypsin used in the passage procedure.

In conclusion, three parameters which are either directly or indirectly implicated in plasma membrane events are altered as cells age *in vitro*. Bradley *et al.*²⁴ have recently determined that only at the last population doubling of WI-38 cells do these cells have increased susceptibility to proteolysis of total proteins. The present results indicate that such proteolysis probably proceeds through the pH 3.4 or other protease activity but not the pH 7.8 protease since more activity remains at the last population doubling. Furthermore the fact the total protein proteolysis does not occur until this last population doubling of WI-38 may indicate this is merely a terminal reaction while surface alterations such as decreased electrophoretic mobility and loss of activity of a potentially critical surface active pH 7.8 protease may indeed initiate these terminal events.

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Voltage sensitivity of acetylcholine currents in *Aplysia* neurones in the presence of curare

At the frog neuromuscular junction, acetylcholine (ACh) induces fluctuations of the membrane potential¹ or current², from which one can evaluate the intensity and the duration of the 'elementary current' flowing through individual ionic channels. The observation that the duration of the elementary event increases with hyperpolarisation³ has provided an ex-

planation for the fact that, when ACh is applied at the end plate in long pulses, the total ACh current increases more with hyperpolarisation than does the elementary current^{3,4}. By studying the currents induced by ACh on *Aplysia* neurones, we have observed a similar phenomenon. But we have discovered that in the presence of curare the total ACh current increases little or even decreases with hyperpolarisation, while the elementary current behaves as in control conditions. The analysis of this phenomenon suggests that, in our preparation, most of the binding of curare does not occur on the free ACh receptor, as in the frog, but rather follows the binding of ACh. It also suggests that the binding of curare is voltage dependent.

We selected for our study a group of small cells (about 60 μm in diameter) situated at the posterior pole of the right pleural ganglion. These cells respond to ACh by a purely excitatory response (completely abolished by hexamethonium 10^{-3} M, see ref. 5). They have a high input resistance (10–40 M Ω) and display very little spontaneous synaptic activity. They were voltage-clamped using two low-resistance (2–10 M Ω) microelectrodes. Temperature was set at 12 °C. ACh was applied iontophoretically from pipettes filled with 1 M AChCl in pulses of about 30 s. Spectrum analysis of the current fluctuations was done on a Hewlett Packard Fourier analyser. Background noise in the absence of ACh was always small enough to make the subtraction of spectra unnecessary.

We first compared the voltage sensitivity of the 'elementary ACh current' to that of the 'total current'. The elementary current, i_{el} , was calculated as the ratio of the variance over the mean of the total ACh current^{1,2}. It increased roughly linearly from –40 to –120 mV (Fig. 1). The total current also increased linearly between these values (Fig. 2), but the increase was larger. For example, between –40 and –80 mV, the elementary current was multiplied by 1.35, whereas the total current was multiplied by 2.5. A clue to this difference was given by the analysis of the ACh noise power spectrum. This spectrum could be fitted with a single Lorentzian component. The average life time of the channels, τ , was calculated from the cut-off frequency, f , as $\tau = 1/2\pi f$ (refs 1 and 2). τ increased with hyperpolarisation: it was about 10 ms at –40 mV and 18.5 ms at –80 mV (Fig. 1). This increase accounts for the difference in the voltage dependences of the elementary ACh current and of the total ACh current.

We then repeated the experiments in the presence of tubocurarine (TC) (5×10^{-5} M). The values of i_{el} and τ were unchanged (Fig. 1). On the other hand, curare drastically modified both the time dependence and the voltage dependence of the

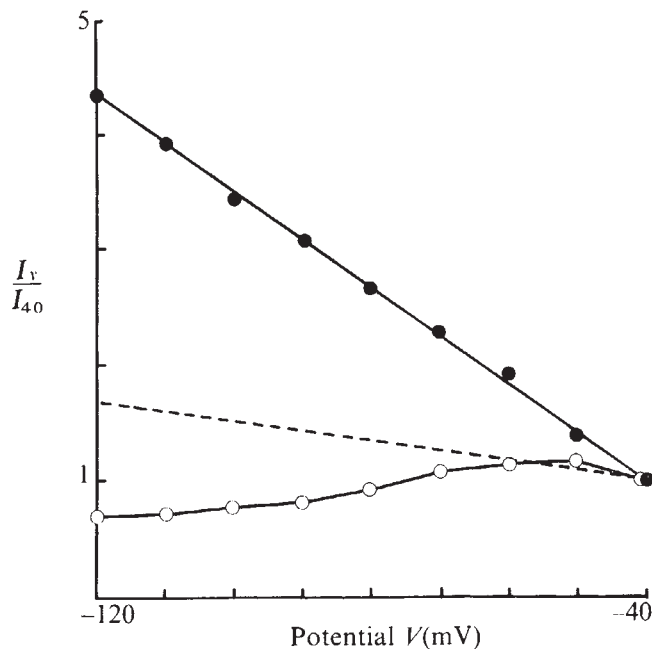


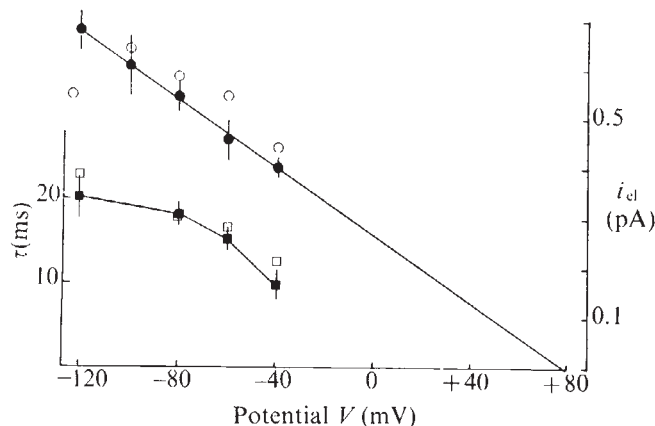
Fig. 2 ACh plateau current as a function of voltage normalised to its value at –40 mV. Control (●): the current increases with hyperpolarisation. In 10^{-4} M TC (○), the current decreases with hyperpolarisation beyond –50 mV. ---, Variation of i_{el} replotted from Fig. 1.

total ACh current. Fig. 3 shows the responses to ACh at two potentials, before and after application of curare (5×10^{-5} M). In normal seawater (Fig. 3a) the response was larger at –80 mV than at –40 mV. In the presence of curare (Fig. 3b), however, the early part of the response increased with hyperpolarisation but the later part was nearly unchanged. The responses of Fig. 3b (in the presence of curare) were obtained with higher doses of ACh than those of Fig. 3a. When the ACh current was not increased after curare treatment comparison of the responses with the controls showed that the initial part of the ACh response was changed little or not at all by curare, which acted only on the later part of the rising phase. This suggests that most or all of the curare blocking action depends upon prior activation of the receptor by ACh.

Experiments like that of Fig. 3 are made difficult by the long recovery periods needed to compare adequately successive ACh responses. Another way of analysing the voltage dependence of the ACh currents is to modify the potential during a given ACh application. Figure 4 shows such an experiment. In control conditions, when the membrane potential was brought from –40 to –80 mV, the ACh current showed a two-step increase: an instantaneous one (expected from the increased driving force), then a more progressive one. Repolarisation brought similarly a two-step decrease. This behaviour, similar to what has been observed elsewhere^{6–8}, is what is expected from the variations of τ with V . More unexpected, however, is the behaviour observed under curare. There (Fig. 4b) when the membrane potential was brought from –40 to –80 mV, the increase in current was followed by a decrease which eventually brought the steady ACh current close to that measured at –40 mV. Fig. 2 shows the voltage dependence of the steady current obtained from such experiments.

The 'curare-induced relaxation' was not of a pure exponential form. It was markedly speeded up as the concentration of curare increased from 10^{-5} M to 2×10^{-4} M. Its kinetics seemed specific for curare, since preliminary experiments showed that voltage-dependent phenomena qualitatively similar to those of Figs 2 and 4 can also be obtained with a variety of cationic blockers of the ACh response (hexamethonium, procaine, atropine, tetraethylammonium) but that, for

Fig. 1 Elementary ACh current as a function of membrane potential in control experiments (●) and in the presence of 5×10^{-5} M curare (○), and average lifetime of the opened channels calculated from noise power spectra in the absence (■) and in the presence (□) of curare. The control points are the mean of 5–19 experiments with error bars corresponding to twice the s.e.m. Points in the presence of curare are the mean of 1–3 experiments.



a given blocking effect, the kinetics of the relaxation varies markedly from one antagonist to the other.

The comparison of our results with those obtained at the frog neuromuscular junction indicates a number of differences.

If one assumes that the linearity of the I - V curves for the elementary current (Fig. 1) is maintained at depolarised membrane potentials, the extrapolation of this curve leads to a reversal potential of +80 mV, and to an elementary conductance of $3.3 \times 10^{-12} \Omega^{-1}$. The value of +80 mV is close to the value of the Na^+ equilibrium potential in *Aplysia* neurones⁹. It is therefore tempting to suggest that the ionic channels opened by ACh in *Aplysia* are pure Na^+ channels, in contrast to the Na^+ - K^+ channels of the frog's endplate. This difference in selectivity could be linked with the smaller value of the elementary conductance in *Aplysia*.

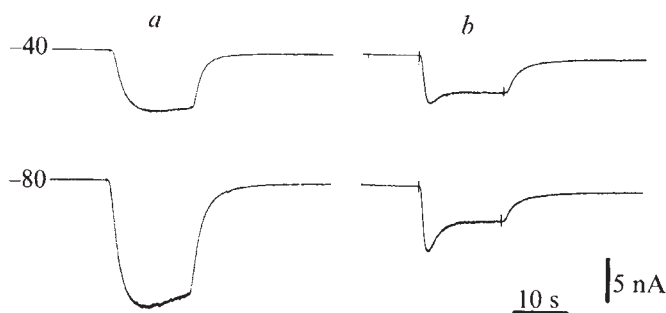
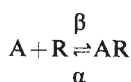
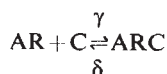


Fig. 3 ACh currents obtained at -40 and -80 mV in control conditions (a) and in 5×10^{-5} M TC (b). The position of the ACh electrode was not changed during the experiment, but the intensity of the iontophoretic current was increased in b. Note the noise increase associated with the ACh current. Calibrations, 5 nA and 10 s.

The receptor linked with the excitatory effects of ACh in *Aplysia* is known to be different from the endplate's receptor⁵ and it is therefore not too surprising that curare does not act as a simple competitive inhibitor of ACh in *Aplysia*. In fact, the effects of curare in *Aplysia* resemble in many respects those of local anaesthetics on the endplate: both the effects on the shape of the ACh response and the reduction of ACh conductance with hyperpolarisation have been observed under procaine or xylocaine in the frog (refs 10-12 and see ref. 13). One interpretation of these effects of local anaesthetics supposes binding of the drug either to an 'activated' ACh receptor complex^{11,12} or to the open channel itself¹⁴. This type of interpretation can be applied to the effects of curare in *Aplysia*. For example, if the reaction of ACh (A) with its receptor (R) is written



where AR corresponds to the active (open) configuration of the ACh receptor complex, then the main action of curare could be written



ARC being assumed to be non-conducting.

One has to account, however, for an important point: procaine modifies the shape of the ACh noise power spectrum in the frog¹⁵ whereas curare has no such effect in *Aplysia* (Fig. 1). The model predicts a shortening of τ in the presence of the antagonist, C. But the amount of shortening will depend

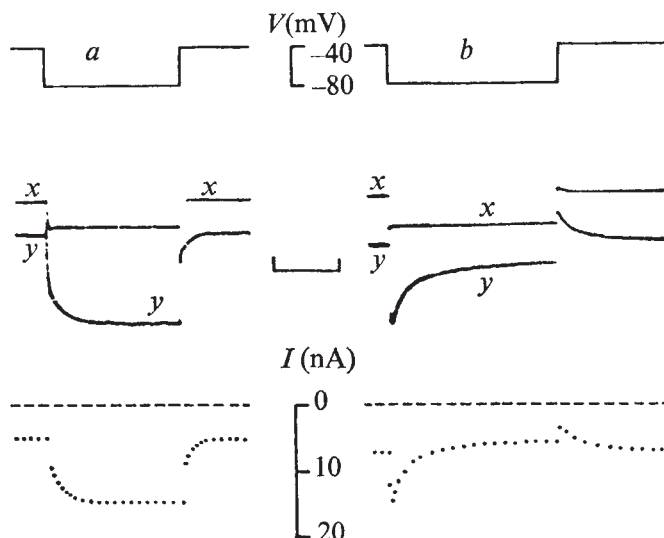


Fig. 4 ACh current response to a voltage step in a control experiment (a) and in 10^{-4} M TC (b) (from two different cells). Top traces: the voltage was stepped from a holding value of -40 mV to a test value of -80 mV and back. Middle traces: currents obtained before (x) and during (y) the application of ACh. Lower traces: ACh current (I_{ACh}) obtained by subtraction of y from x. As in Fig. 3, inward currents are downwards. Time calibration: 100 ms for a; 2 s for b. A relaxation in the same direction as in a can barely be seen at the beginning of the current at -80 mV in b; with a faster sweep speed, it was observed to be very similar to the relaxation seen in a. The relaxations in a could be fitted with simple exponentials. The time constants of these exponentials (11 ms at -40 mV; 17 ms at -80 mV) compare well with the values of τ obtained from noise analysis (see Fig. 1).

on the relative values of γ , $[\text{C}]$ and α , assuming that both β , $[\text{A}]$ and δ are small compared to α . Thus, if the rate constants are such that in our experiments

$$\gamma \cdot [\text{C}] \ll \alpha$$

curare will not modify τ appreciably.

We can also account for the fact that the blocking effect of curare increases with hyperpolarisation by assuming, in the above model, that the binding of curare on AR is favoured by hyperpolarisation. Such voltage sensitive binding¹² could occur if the binding site for curare were somewhat inside the hydrophobic part of the membrane, in a region sensitive to the externally applied potential.

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Strontium-calcium substitution in synaptic transmission in turtle retina

RECENT electrophysiological and morphological studies strongly suggest that the synaptic transmission between photoreceptors and horizontal cells in the vertebrate retina is chemical in nature, although the anatomical appearance of the neuronal contacts does not strictly conform to that of conventional chemical synapses¹⁻³. It has been shown that changes in the ionic composition of the extracellular medium, such as lowering calcium, increasing magnesium or adding cobalt, that in the chemical synapses are known to block the release of transmitter^{4,5}, abolish the response to light of horizontal cells without reducing that of photoreceptors⁶⁻⁸. The present report provides additional evidence that the synapses between photoreceptors and horizontal cells have properties characteristics of conventional chemical synapses. It is known that in the chemically transmitting synapse, strontium ions can substitute calcium ions in the process of transmitter release⁹⁻¹¹. I have studied the effects of calcium-strontium substitution on the intracellularly recorded responses to light of horizontal cells and cones in the retina of the turtle (*Pseudemys scripta elegans*).

After draining the vitreous humour, the isolated eyecup, with the retina still attached to the pigmented epithelium, was mounted in a perfusion chamber¹² where a modified Ringer solution continuously flowed over the vitreous side¹³. Intracellular recordings were made with glass micropipettes filled with 4 M potassium acetate. The light stimuli were the reduced images of circular apertures, the diameter of which on the retina could be varied from 1,200 to 100 μm . The light source was a quartz iodine lamp (Riluma 100 W) and neutral density filters were used to reduce light intensity. The total flux density of unattenuated light at retinal level was about $1.2 \times 10^4 \text{ erg cm}^{-2} \text{ s}^{-1}$ with wavelengths from 450 to 900 nm. Receptor and horizontal cell responses were identified by the criteria given by Baylor and Fuortes¹⁴.

The effects of calcium-strontium substitution on the responses to light of a horizontal cell are shown in Fig. 1. Adding Sr^{2+} to the retinal medium restores the light response of the cell abolished by calcium removal (Fig. 1A). The shape of transients at the light onset and offset, however, differs in the presence of Sr^{2+} with respect to the control Ringer (Fig. 1B). Similar changes are observed in the depolarising transient at the light offset, both in strontium and EDTA Ringer, and so they seem to depend on the

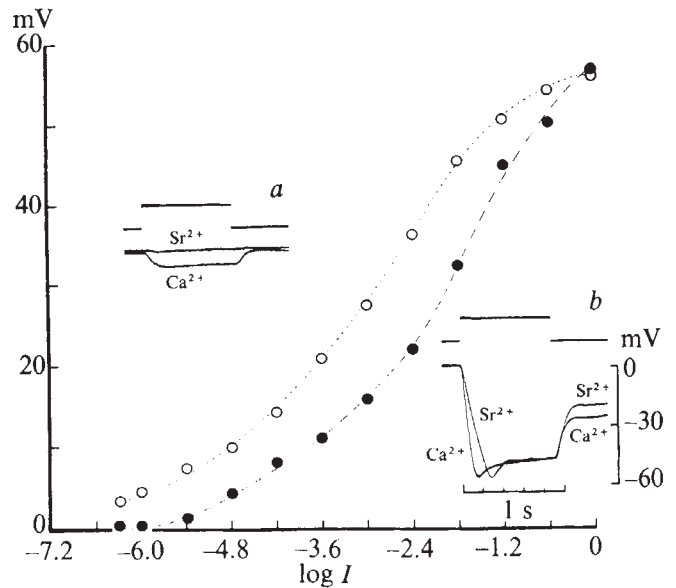


Fig. 2 Peak amplitude of the response of a horizontal cell in control Ringer ($\circ$) and during perfusion with calcium-free strontium solution ($\bullet$), plotted as a function of light intensity. Records in insets *a* and *b* are the responses obtained in the two conditions to light attenuated by 5.4 log units (*a*) and to the maximum available energy (*b*). Area illuminated was 1,200 μm in diameter.

absence of calcium ions, while the prolongation of the depolarising phase at the light onset is seen only in the absence of strontium. Further differences between the response to light of horizontal cells in control Ringer and in calcium-free strontium solution, are illustrated in Fig. 2, in which the stimulus response functions ($V/\log I$) are plotted for the same horizontal cell in the two conditions. The amplitude of the response to light in the presence of Sr^{2+} is lower than that in control Ringer over most of the intensity range (see in particular inset *a*) but approaches the control amplitude at the highest intensity (see inset *b*).

These differences in the response of the horizontal cell can be interpreted as being due, at least in part, to the action of the ionic changes on the responses of presynaptic neurones—that is, cones. Indeed, control experiments have shown that during the perfusion with low calcium-strontium solution the $V/\log I$ curves of the cones and their depolarising transients at the end of the stimulus are modified in a way comparable to that observed in the horizontal cells. Similar modifications are also observed during perfusion with low calcium-EDTA Ringer, again suggesting that they

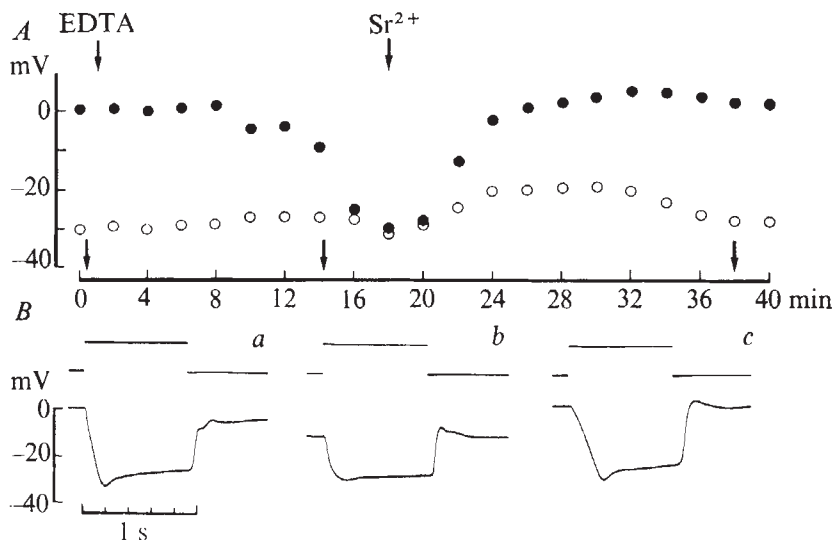


Fig. 1 *A* and *B*, Effects of calcium-strontium substitution on response to light of a horizontal cell. *A*, $\bullet$, Level of membrane potential in darkness; $\circ$, peaks of responses to light. *B*, Zero level of membrane potential is arbitrary and indicates the level of membrane potential in darkness during the perfusion with control Ringer. During the experiment the retina was stimulated with long pulses of light (see the trace above each record in *B*) at a constant rate (0.1 s^{-1}). Light intensity was attenuated by 2.4 log units with respect to the maximum available energy; the area illuminated was 1,200 μm in diameter. Arrows on abscissa in *A* indicate the time at which the records in *B* were taken. EDTA indicates the application of a Ringer solution without calcium containing 2 mM l^{-1} of EDTA, and Sr^{2+} the application of a Ringer solution without calcium, containing 2 mM l^{-1} of strontium ions.

depend on the absence of calcium ions from the medium rather than on the presence of strontium (see also ref. 8). On the contrary, the time course of the hyperpolarising transient of the cones at the onset of the stimulus is not significantly different in the three conditions.

Previous work has shown that the transmission of electrical signals from photoreceptors to horizontal cells depends critically on the presence of calcium in the extracellular fluid⁷ and is blocked either by excess magnesium⁶⁻⁸ or by the presence of cobalt ions⁷. In this paper I have demonstrated that the addition of strontium ions to the retinal medium restores the response of horizontal cells to light abolished by calcium removal. All these properties are characteristic of the chemical transmission in conventional synapses and therefore these results strongly support the chemical nature of the signal transmission from photoreceptors to horizontal cells.

As to the modifications in the time course of the depolarising transient and in the $V/\log I$ curves observed in the response of the horizontal cells during the perfusion with calcium-free strontium Ringer, these seem to depend on similar changes in the behaviour of the cones in the same conditions. On the contrary, the prolongation of the time course of the hyperpolarising onset in the horizontal cells responses has no counterpart in cone responses. At the neuromuscular junction Sr^{2+} ions are known to increase, by way of a postsynaptic effect, the duration of transmitter action. If one assumes that the hyperpolarising responses to light of horizontal cells result from a reduction in the release of a depolarising transmitter¹³ continuously flowing in darkness from the receptor pedicle, increasing the duration of transmitter action would result in a slower time course of the hyperpolarising phase at the light onset.

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Postexposure serum prophylaxis of neonatal herpes simplex virus infection of mice

PERINATAL infections with herpes simplex virus (HSV) cause death or permanent disability in most reported cases¹⁻⁵. In theory, control of this disease by immunotherapy might be feasible, much like postexposure serum prophylaxis of rabies, since the time of HSV infection in the birth canal is usually known and antibody can be administered soon thereafter. Treatment with antibody has not been encouraged, however, because (1) many investigators consider this immunity cell-mediated rather than humoral⁶⁻¹¹; (2) effective administration of human immune globulin in neonatal herpes of man has not been demonstrated^{1,3,12}, and (3) transplacental antibody conferred only partial protection in human neonatal infections¹. There is further discouragement in animal studies which failed to show protection by antibody⁹⁻¹¹, although antibody was

protective in some animal studies¹³⁻²⁰. The findings reported here raise again the possibility that antibody treatment might be effective in specific conditions.

The concept that cell-mediated immunity, rather than humoral immunity, is the major host defence has been based in part on increased severity of HSV infection after impairment of T-lymphocyte function⁶⁻¹¹. But, findings that HSV antibody production is T-cell dependent^{9,20,21} suggest that exacerbation of some of these infections by impaired T-lymphocyte function could have been due to decreased antibody formation²⁰. Furthermore, protection of mice that received syngeneic, immune spleen cells and were infected with HSV might, in some cases, be due to the transfer of antibody-producing or helper cells, since high levels of antibody were produced more rapidly in these mice than in control mice (unpublished observations). Consistent with this view is the protection of nude²⁰, immunosuppressed, and normal mice (unpublished observations) against HSV infection by sufficiently high levels of passively administered antibody and apparent protection against HSV by maternal antibody of infants (excluding newborns) under 11 months of age²².

Re-examination of antibody potential in a model of neonatal HSV infection is indicated by these findings, even though they do not establish the importance of antibody in all conditions. For example, strains of HSV that spread principally through the blood stream might be affected by antibody, but not strains of HSV that are transmitted through nerve pathways which exclude antibody.

Our study was designed to determine whether administration of various units of neutralising antibody could achieve post-exposure prophylaxis of experimental neonatal HSV infection²⁰. Mice (Swiss, CDF-1 or BALB/c), age 1-4 d, were infected subcutaneously with from 1 to 300 newborn mouse LD₅₀ of HSV, either type 1 (VR3 strain) or type 2 (MS strain). One hour after infection, the experimental mice were injected intraperitoneally with 0.1 or 0.2 ml of type-specific hyperimmune rabbit antiserum. Controls were injected with normal rabbit or foetal bovine serum, or Eagle's tissue culture medium containing 2% foetal bovine serum. Mice were observed for at least 12 d. Percentage protection was calculated as follows:

$$\frac{\% \text{ mortality of controls} - \% \text{ mortality of treated}}{\% \text{ mortality of controls}} \times 100$$

Rabbit antisera were prepared by initial injections of HSV virus either type 1 or type 2 (grown in rabbit kidney cultures and emulsified with Freund's incomplete adjuvant) into the footpads and back (0.25 ml containing 10^{5.4} PFUs of HSV), followed by four to eight weekly subcutaneous and intravenous injections of virus without adjuvant.

Protection consistently occurred in newborn mice infected with 1-20 LD₅₀ of either type of HSV and treated with a total of 12,000 U of antibody (Table 1). No protection occurred when the large dose of antibody was administered to mice infected with a high challenge dose (100-300 LD₅₀) of HSV. Little or no protection followed treatment with 600 U of antibody. Better results attained against HSV type 1 warrant further study.

Our findings demonstrate good protection against experimental neonatal HSV infection by large doses of antibody in mice infected with low challenge doses of virus. Failure of antibody in some animal studies⁹⁻¹¹, but not in others¹³⁻²⁰, could conceivably be due to such variables as antibody dose¹⁷, virus challenge dose¹⁴, portal of entry HSV, and difference in pathogenesis, depending on the virus strain used. These same factors might account for the reported failures of human immune globulin to protect newborn infants against HSV infection^{1,3,12}. Additional study of these and other variables may further determine the optimum conditions for antibody protection of newborns.

Although our results were obtained using mice infected subcutaneously, other studies have shown antibody protection

Table 1 Protection by antiserum of newborn mice against HSV infection

Expt	Mouse strain (Age, d)	HSV type (strain)	Challenge dose (LD ₅₀)	Dose of antibody (U per mouse)	Dead mice/ Total mice	% Mortality	% Protection	Combined% protection*
1	CDF-1 (1)	1 (VR3)	10 10	0 12,000	7/7 1/8	100 13	87	
2	Swiss (1)	1 (VR3)	2	0 12,000	6/9 0/10	67 0	100	
3	Swiss (1)	1 (VR3)	20	0 12,000	19/21 1/19	90 5	94	94†
4	CDF-1 (1)	1	100 100	0 12,000	6/7 6/7	86 86	0	0
5	BALB/c (1)	2 (MS)	2	0 600 0 600	3/8 5/5 7/7 7/7	38 100 100 100	0 0	
6	Swiss (1)	2 (MS)	20	0 600	18/22 9/22	82 41	50	18
7	Swiss (4)	2 (MS)	1	0 12,000	3/5 0/6	60 0	100	
		2 (MS)	3	0 12,000	5/5 4/8	100 50	50	
8	Swiss (2)	2 (MS)	2	0‡ 12,000	3/5 2/8	60 25	58	
9	Swiss (1)	2 (MS)	3	0 12,000	5/5 2/6	100 33	67	
10	Swiss (1-2)	2 (MS)	3	0‡ 12,000	7/8 2/14	88 14	84	71†
11	Swiss (1-3)	2 (MS)	300 300	0 12,000	18/18 25/25	100 100	0 0	0

* Average % protection for preceding experimental group.

† $P < 0.005$.

‡ Normal rabbit serum.

of newborn or young animals infected intranasally^{13,19}, intradermally¹³, intracerebrally^{14,16,17}, intraperitoneally¹⁴, intravaginally¹⁸ or subcutaneously¹⁶. Because of these earlier findings, human immune globulin treatment in man had been considered^{1,12,19,24}. If proper conditions can be defined to protect newborn infants with hyperimmune human globulin, this treatment would offer several advantages over present methods: for example, administration of human immune globulin to newborns is much safer than surgical delivery of the babies or than use of toxic drugs. Finally, combination of protective antibody with other antiviral agents, such as interferon, might enhance protection as with vaccinia virus infection of mice²³.

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Type C virus in lymphosarcoma in northern pike (*Esox lucius*)

THE northern pike (*Esox lucius*) is a highly prized freshwater fish, both as a game fish and as a commercial species. Epizootics of lymphosarcoma occur widely in North American pike¹ and in the Old World^{2,3}. The tumour in pike has been found with an overall frequency of 20.9%, which is the highest frequency of a malignant neoplasm in any known free living vertebrate¹. All pike with the tumour have cutaneous lesions, and epizootiological evidence suggests that the disease is transmitted horizontally by contact during spawning¹. The tumour is transplantable and evidence of cell-free transmission has been found⁴. In an attempt to resolve the aetiology of the disease, we have investigated the presence of oncornavirus in pike lymphoma. Since all known RNA tumour viruses possess the enzyme reverse transcriptase, we have sought the activity of this enzyme in post-mitochondrial particulate fractions prepared from pike lymphoma tissue.

Pike lymphomas were analysed as described in the legend

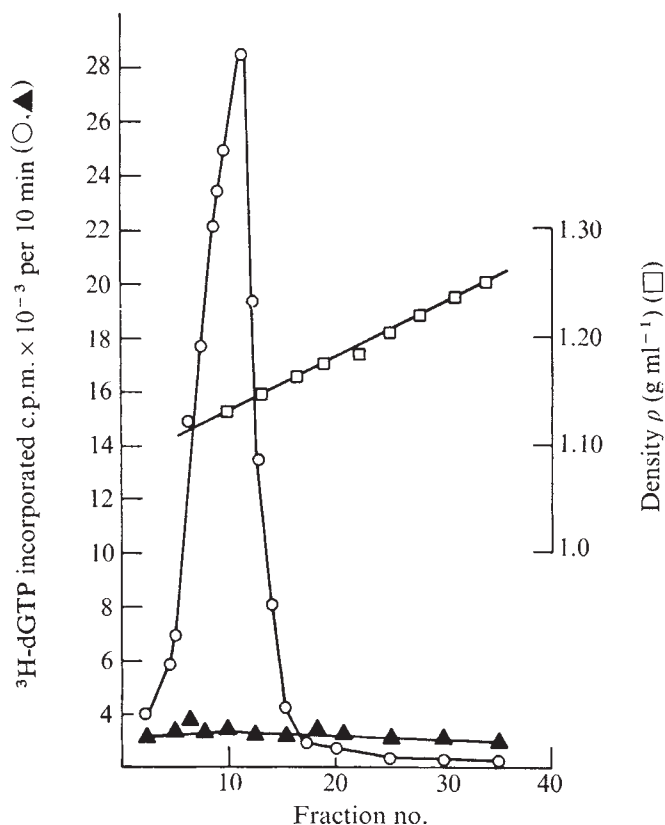


Fig. 1 Identification of the density of reverse transcriptase in a postmitochondrial extract of pike lymphoma by sucrose gradient centrifugation. Frozen pike lymphoma tissue (10 g) was homogenised at 4 °C in 2 volumes of 0.05 M Tris-HCl (pH 7.5), 0.01 M dithiothreitol and 0.25 M sucrose. It was then sonicated for 1 min and the suspension was centrifuged at 1,000g for 30 min at 4 °C. The supernatant was centrifuged at 16,000g for 30 min. The supernatant was again centrifuged at 100,000g for 1 h at 4 °C in an SW27 rotor. The resulting pellet (P-100) was suspended in 0.5 ml of 0.01 M Tris-HCl (pH 7.5) containing 0.01 M dithiothreitol and layered in a 13 ml 25–60% (w/v) sucrose gradient in TNE and centrifuged at 100,000g for 22 h in an SW41 rotor. Approximately 40 fractions were collected from the top by Buchner densiflow. A sample (20 µl) was added in a reaction mixture of (100 µl) containing 100 mM Tris-HCl (pH 8.3), 50 mM KCl, 1 mM MnCl₂, 4 mM dithiothreitol for poly(Cm).oligo(dG) (20 µg ml⁻¹) and 0.125 M ³H-dGTP. Reaction mixtures were incubated at 20 °C for 1 h and stopped by addition of 10% trichloroacetic acid (TCA). Acid precipitable material was collected on Whatman GF/C filters and the radioactivity was counted. Normal pike tissue was processed in identical conditions and assayed as described above. ○, Pike lymphoma; ▲, normal pike.

to Fig. 1. A cytoplasmic particulate fraction was isolated from the tissue. After further fractionation on sucrose gradients, fractions were assayed for the presence of reverse transcriptase able to utilise poly(Cm)-oligo(dG), a template primer specific for viral polymerases⁵. Reverse transcriptase activity was associated principally with the particulate fraction sedimenting at a density of 1.16 g cm⁻³. Parallel gradients run with purified Rauscher leukaemia virus were fractionated and assayed similarly for reverse transcriptase activity. Peak activities also occurred at a density of 1.16 g cm⁻³. When normal pike tissue was analysed identically, no reverse transcriptase activity was detected in any gradient fraction. Thus the reverse transcriptase activity was unique to the lymphoma, and was associated with a particulate fraction corresponding to the density of known RNA tumour viruses.

The seasonal nature of the appearance of the pike lymphoma prompted us to examine the temperature profile of the reverse transcriptase activity. As Fig. 2 shows, the enzyme activity from pike lymphoma exhibited 82% of its

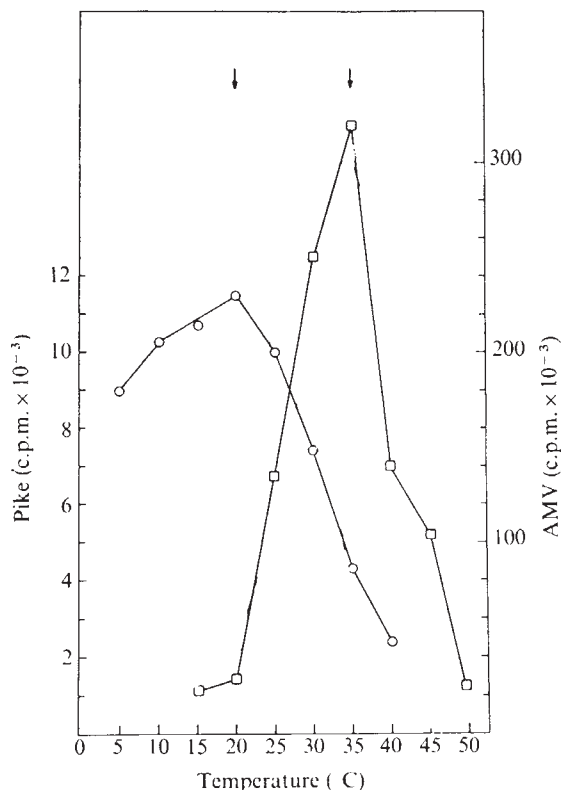
maximum activity at 5 °C, with an optimum at 20 °C while only 35% was retained at 35 °C. In contrast, reverse transcriptase purified from avian myeloblastosis virus (AMV) has a temperature optimum of 35 °C with only 0.6% of optimal activity at 20 °C and no detectable activity at 5 °C.

Fractions between 1.15 and 1.17 g cm⁻³ were diluted to less than 10% sucrose, pelleted and processed for electron microscopy. Figure 3 shows the appearance of the numerous type C virus-like particles present in these gradient fractions. Most particles appear similar to typical type C virus particles of avian and mammalian origins, but a more careful categorisation must await the detection of budding and immature forms of the virus.

The results reported here demonstrate that pike lymphoma contains poly(Cm)-oligo(dG)-directed DNA polymerase activity present in a structure with the density of an RNA tumour virus. These particles are not disrupted by physical manipulation and maintain their characteristic density when centrifuged repeatedly. This is the first report of the presence of reverse transcriptase in a fish, which is phylogenetically the most primitive animal in which the enzyme has been detected.

The aetiological significance of the type C virus-like particles and its associated reverse transcriptase is unknown. The disease in nature exhibits marked seasonal periodicity, with tumours developing during the cold water periods of fall and winter¹, when mean water temperatures are 12 and 4 °C, respectively. Field epizootiological studies have revealed that the tumours frequently regress spontaneously during the summer months¹, with typical water temperatures varying from 21 to 30 °C. The high activity of the enzyme at low temperatures correlates with the water tem-

Fig. 2 Temperature profiles of partially purified pike and purified AMV reverse transcriptase. Samples were incubated for 30 min in the corresponding reaction mixtures at the temperatures indicated. Reaction mixture temperatures were accurately controlled by using a Lauda circulating water bath. Reactions were stopped by addition of cold 10% TCA, and amounts of ³H-dGMP incorporated into acid-insoluble product was determined. ○, Pike; □, AMV.



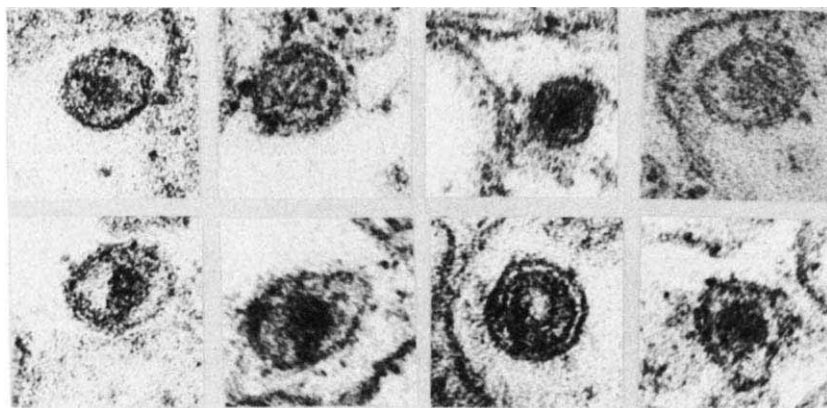


Fig. 3 After frozen tumour had been thawed, homogenised, and fractionated on a sucrose gradient, fractions with densities between 1.5 and 1.17 g cm⁻³ were pooled, diluted and pelleted at 100,000g. The pellet was fixed for 2 h in 3% glutaraldehyde in Milling's buffer, washed for 30 s in buffer, post-fixed for 1 h in 1% osmium tetroxide, washed for 2 h in distilled water, stained *en bloc* with 2% uranyl acetate in 50% ethanol, dehydrated in ethanol and propylene acids, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined at 80 kV in a Siemens 101 electron microscope ($\times 160,000$).

peratures at which the tumour develops in nature. The warm water period of summer (non-permissive temperatures) may explain the seasonal periodicity of the disease and the mechanism of the spontaneous regressions observed. The disease in fish offers a unique probe, facilitating both *in vivo* and *in vitro* assays to resolve 'turn on' and control mechanisms of oncornavirus-induced transformation, as both cell cultures and hosts can be placed at permissive and non-permissive temperatures.

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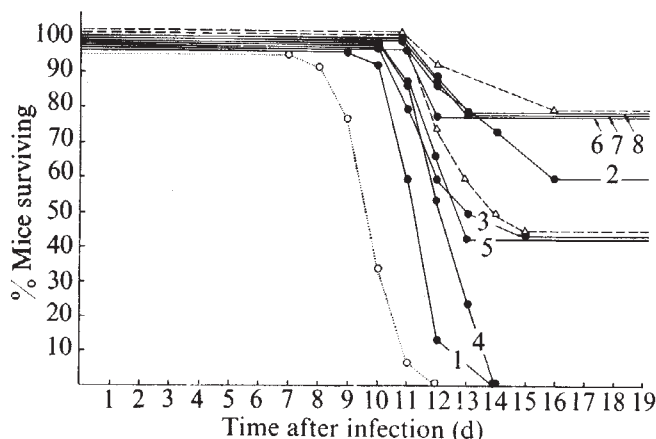
Virulence of different strains of *Toxoplasma gondii* and host response in mice

THE protozoan *Toxoplasma gondii* has a world-wide distribution. It is an obligate intracellular parasite and appears in many species of mammals and birds. Animals that survive the acute stages of *Toxoplasma* infection probably remain infected for life¹. The RH strain of *Toxoplasma gondii*, originally isolated by Sabin² and widely used in the routine Dye test³ is extremely pathogenic to mice, LD₁₀₀ by intraperitoneal infection being below 10 tachyzoites⁴. Moreover, it is difficult to obtain survival after RH challenge of mice immunised with a strain of lower virulence⁵. Such survival without the use of chemotherapy has, however, been reported^{1,6,7}. The RH strain was not totally eradicated⁷ from immunised mice even 7 weeks after challenge. How do immunised mice suppress the RH strain—by total elimination or making it possible for the RH strain to remain in the body as does the immunising strain? Furthermore, in the latter case, would the inherent virulence of the RH strain become changed? We here report that *Toxoplasma* possessing the full virulence of the original RH strain, could be isolated from mouse brains 14-18 months after RH challenge of mice which had first been immunised with the Beverley strain. This applied, however, to a proportion of

the mice; in the brains of the other mice only the immunising strain (Beverley) was found.

Brain suspensions from test groups (mice infected with the Beverley strain and reinfected with the RH strain) were injected subcutaneously into normal mice (Fig. 1). In two of the test groups (1 and 4) all mice died as did the RH controls. In the others a varying proportion of the mice succumbed, an event which also occurred in Beverley infected controls. In this comparison the RH control infection started from the tachyzoite stage, whereas the others started from a cystic stage. To establish a comparison with all infections arising from the tachyzoite stage, the following procedure was undertaken: Two mice from each of the test groups 1, 2 and 3 as well as the RH and Beverley controls were killed after 6-9 d, brain suspensions were made and injected into groups of normal mice (Fig. 2). In test groups 2 and 3 all mice survived as did the Beverley controls. The surviving mice had become Dye test-positive, proving that they had gone through a *Toxoplasma* infection. In test group 1 all mice died, the survival time being almost identical to that found for RH-infected mice, indi-

Fig. 1 Survival rates of mice after infection with *Toxoplasma gondii*. Brain suspensions of mice (female NMRI/Bom) which had been infected with *Toxoplasma gondii* were injected into normal mice. Brains were derived from mice which had been infected in three different ways. ●, Test groups. Mice which had been infected first with the Beverley strain and then with the RH strain (5×10^3 or 5×10^6 tachyzoites intraperitoneally) 2 weeks-4 months later. 14-18 months after the RH challenge the brains were dissected out. Number of the individual test group as shown. △, Mice which had been infected merely with the Beverley strain 12 months previously. ○, Mice with a RH infection of 4 d duration. Brains were mortared in 0.9% phosphate-buffered saline. Each brain suspension to be tested was prepared from one mouse or from two parallel mice. The brain suspensions were diluted so that one brain would provide enough material for infection of 20 new mice. Aliquots (0.3 ml) of the brain suspensions were injected subcutaneously to each normal mouse (9-20 mice in each group).



cating that we were dealing with the RH strain in test group 1, but not in test groups 2 and 3.

Further support was obtained from experiments in which the brain suspensions from test groups and controls were examined with regard to their ability to produce peritoneal exudate. The virulent RH strain is much more efficient than the Beverley strain in this respect. In test groups 1 and 4 a large amount of peritoneal exudate (about 1 ml) was found in all five mice tested. In test group 5 two out of five mice showed this marked exudate production. In the other test groups only small amounts of exudate (about 0.1 ml) were found. A direct comparison of the virulence

Furthermore, in these groups all mice tested for survival died. In the other test groups all mice survived and only small amounts of peritoneal exudate were found. The surviving mice had all produced antibodies against *Toxoplasma*, as demonstrated by the Dye test. Thus *Toxoplasma* isolated from test groups 1, 4 and 5 behaved exactly like the RH strain.

From these experiments the following conclusions can be drawn. Mice surviving a Beverley infection continue to carry the parasite in their brains. These mice have the ability to survive a consecutive RH infection. In such double-infected mice the RH strain can be found in the brains of some of the mice 1.0–1.5 yr later. Once outside the brains, the strain is as virulent as the original, routinely transferred RH strain. Even though we did not find the RH strain in the brains of the major proportion of the mice, the possibility still exists that it could be present at other sites, since *Toxoplasma* has been demonstrated to form cysts in a number of organs^{8,9}.

From this model, which could be expected to have relevance for other mammals, some interesting aspects arise. A person infected with a strain of low virulence and later with a more virulent strain, may benefit immunologically from the first infection in the extent to which acute disease may be prevented. If the virulent strain remains in the body and keeps its inherent virulent potentiality, however, such a person might under certain circumstances be more threatened than if he had been harbouring a strain of low virulence. First, the possibility exists that the virulent strain could evoke disease from organs not well protected by the immune apparatus—for example, chorioretinitis. Second, during immunosuppressive therapy or debilitating disease a virulent strain might be more likely to be reactivated than a strain of lower virulence.

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Rates of oxygenation and Bohr shift of capillary blood in chick embryos

THE effect of carbon dioxide on the affinity of red blood cells (RBC) for oxygen was first described by Bohr, Hasselbalch and Krogh¹. The higher the P_{CO_2} and/or the lower the pH, the lower the O_2 affinity. The rate of the affinity change, that is, the Bohr shift, has been measured by several investigators^{2–4} by abruptly changing the P_{CO_2} of a suspension of RBC using a rapid flow reaction apparatus combined with a P_{O_2} electrode. They showed that the Bohr shift was much slower than the simple oxygenation and deoxygenation rates. While developing a microphotometer technique for kinetic studies of a single RBC in the presence of O_2 and CO (refs 5 to 8), we also attempted to determine the rate of the Bohr shift in the capillary blood of chick embryos, and found that the Bohr shift rate was slower than the O_2 reaction rates; in particular, in the cooperative reactions of O_2 and Bohr shift, the O_2 reaction appeared first followed by the Bohr shift.

Figure 1 represents the O_2 dissociation curves of 16-d-old

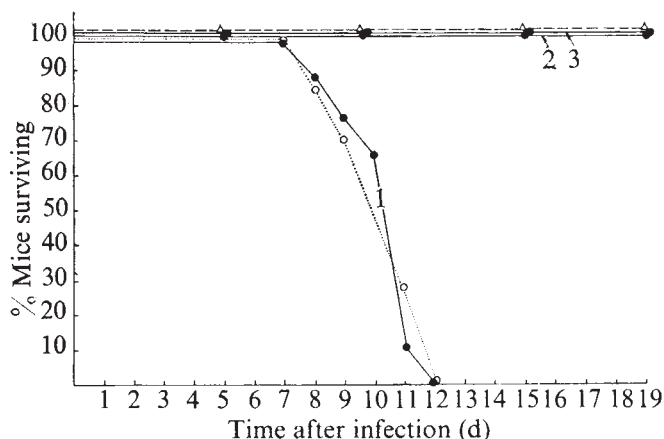


Fig. 2 Survival of mice after infection with *Toxoplasma tachyzoites*. Two of the mice in each group infected with brain suspensions from test groups 1, 2, 3 and the controls (see Fig. 1) were killed after 6–9 d. Brains were mortared in saline and diluted as described in Fig. 1. These brain suspensions were injected into groups of normal mice (7–15 mice in each group). Symbols as in Fig. 1.

of the tachyzoites isolated in the different groups was then carried out. By dilution a low dose (5×10^2 tachyzoites per ml) was prepared in each group and aliquots of 1 ml of these suspensions were injected intraperitoneally to normal mice (Table 1). Tachyzoites of test groups 1, 4 and 5 were as efficient in producing peritoneal exudate as the RH strain.

Table 1 Virulence of isolated *Toxoplasma gondii*

Donor mice	Recipient mice		Survival time (d)
	Production of peritoneal exudate	No. of extracellular tachyzoites (per mouse)	
	Volume (ml)		
Test group No. 1	Mean $\pm$ 1 s.e.	Mean $\pm$ 1 s.e.	Mean (range)
4	1.0 ± 0.1	$28 \pm 4 \times 10^6$	7.4 (7.0–8.0)
5	0.9 ± 0.1	$19 \pm 3 \times 10^6$	7.8 (7.5–8.0)
6	1.2 ± 0.1	$24 \pm 3 \times 10^6$	7.6 (7.0–8.5)
7	0.1	$18 \pm 5 \times 10^3$	All survived
8	0.1	$14 \pm 9 \times 10^3$	All survived
RH control	0.1	$10 \pm 3 \times 10^3$	All survived
Beverley control	0.9 ± 0.1	$29 \pm 7 \times 10^6$	7.1 (6.5–8.0)
control	0.1	$10 \pm 4 \times 10^3$	All survived

The peritoneal exudates isolated from test groups and from controls were centrifuged at 65g for 5 min to sediment the cells. The supernatants containing the tachyzoites were diluted in phosphate-buffered saline (pH 7.2) to give 5×10^8 tachyzoites ml⁻¹. Aliquots (1 ml) of these suspensions were injected intraperitoneally to normal mice (that is, recipient mice). Five mice in each group were used to evaluate survival times and five other mice to evaluate production of peritoneal exudate. The latter mice were killed after 5 d infection, the volumes of the peritoneal exudate which could be aspirated were measured, and the numbers of extracellular tachyzoites in the exudates were recorded by counting in a haemocytometer.

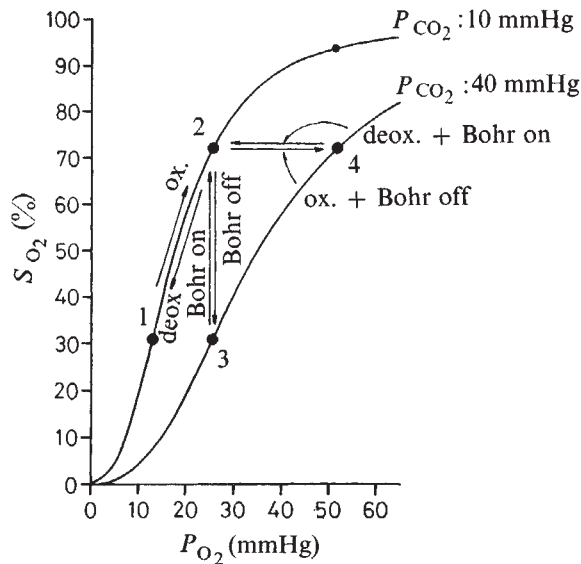


Fig. 1 The oxygen dissociation curves for the capillary blood of 16-d-old embryos determined with the microphotometric apparatus. The closed circles on the curves indicate the reaction gas mixtures used. Gas mixture 1, 13 mmHg P_{O_2} and 10 mmHg P_{CO_2} ; 2, 26 mmHg P_{O_2} and 10 mmHg P_{CO_2} ; 3, 26 mmHg P_{O_2} and 40 mmHg P_{CO_2} ; and 4, 52 mmHg P_{O_2} and 40 mmHg P_{CO_2} . The changes of gas mixtures are shown by arrows.

chick embryos⁹, where P_{O_2} at 50% O_2 saturation (P_{50}) is 17.5 and 35 mmHg for 10 and 40 mmHg P_{CO_2} , respectively. As the P_{CO_2} is decreased from 40 to 10 mmHg, the curve of 40 mmHg P_{CO_2} shifts to the left. Thus, when the P_{O_2} is kept constant, for example at 26 mmHg, the O_2 saturation (S_{O_2}) increases from point 3 to point 2 on the curves in Fig. 1. On the other hand, when the P_{CO_2} is kept constant at 10 mmHg and P_{O_2} is increased from 13 to 26 mmHg, oxygenation occurs and the S_{O_2} increases from point 1 to point 2. Now, if the O_2 diffusion is assumed to be fairly slow compared with the shift of the O_2 dissociation curve, the increase of S_{O_2} in the above two cases should occur at the same rate, because the S_{O_2} values of points 1 and 3 are identical and the P_{O_2} gradients driving the oxygenation are equal to each other. Moreover, when the P_{O_2} and P_{CO_2} are varied simultaneously from 13 to 26 mmHg and from 10 to 40 mmHg respectively (or from 26 to 52 mmHg and from 40 to 40 mmHg respectively), no change in S_{O_2} should occur.

To clarify these speculations we attempted to measure the rate of the S_{O_2} change of RBCs in the chorioallantoic membrane of 16-d-old chick embryos. The membrane forms a single layer capillary plexus of 6 to 10 μ m thickness which is a gas exchange organ of the embryo^{10,11}. The change in S_{O_2} of a single cell in the capillary was measured through the membrane with a microphotometer^{5,6}. The membrane was placed in a small air-tight reaction cuvette and the compositions of O_2 and CO_2 around RBCs were changed stepwise by flushing gas mixtures through the cuvette one after another. The gas mixtures used are numbered on the curves in Fig. 1. The additional gas mixtures 0 and 5 have 0 mmHg P_{O_2} and 300

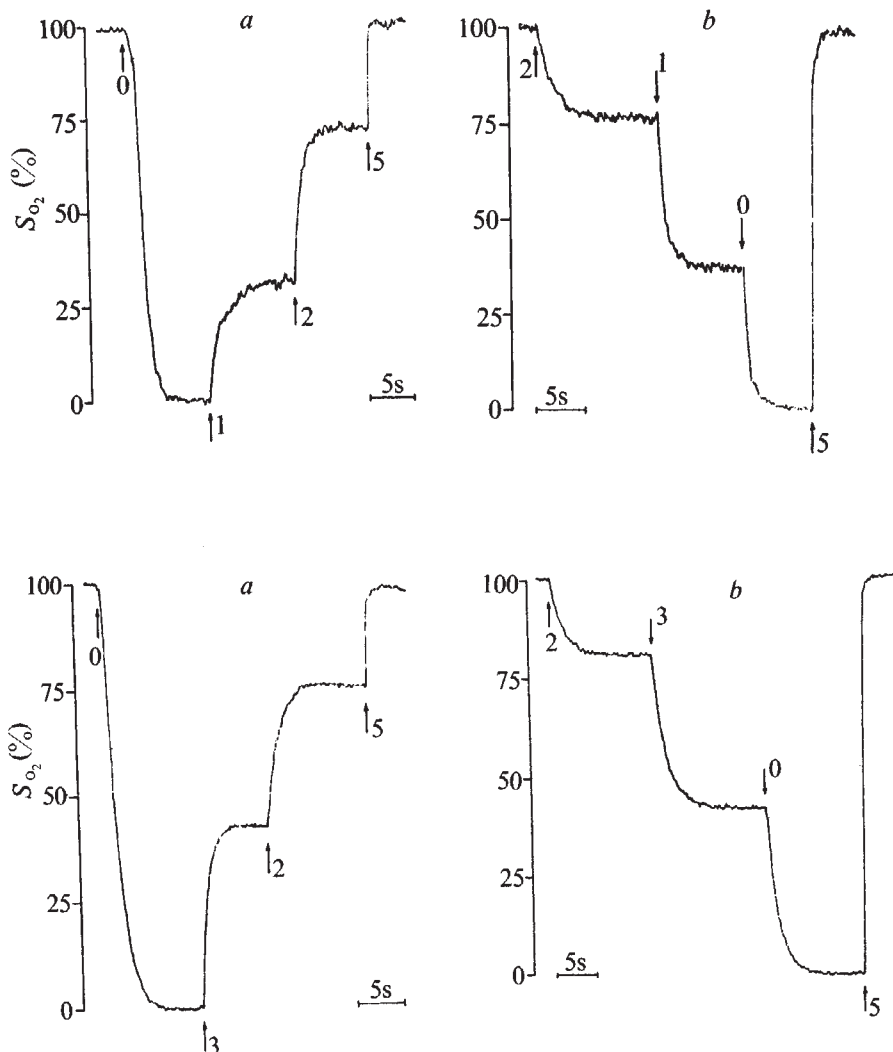


Fig. 2 The O_2 reaction of capillary blood. *a*, Oxygenation reaction. The blood was first deoxygenated completely by flowing the O_2 -free gas mixture (0). The colour change of the RBCs which accompanies the O_2 reaction was detected by a couple of photomultipliers with reference filters of 402 and 418 nm wavelengths^{5,6}. After equilibration, the second gas mixture (1) was flushed through. The oxygenation reaction was then recorded by flowing the third gas mixture (2), and the fourth gas mixture (5) was finally used to attain 100% S_{O_2} state. *b*, Deoxygenation reaction.

Fig. 3 The Bohr shift reaction. *a*, The Bohr on-shift reaction made by a sudden change in P_{CO_2} from 40 to 10 mmHg keeping P_{O_2} constant (26 mmHg). *b*, The Bohr off-shift reaction.

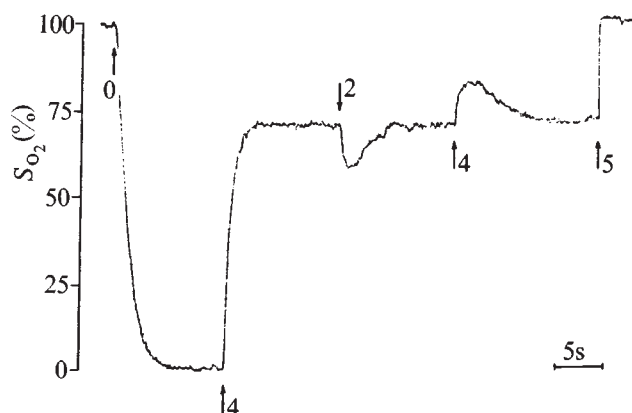


Fig. 4 The cooperative reaction of O_2 and CO_2 . After complete deoxygenation, the blood was equilibrated with gas mixture 4. In the cooperative reaction of O_2 and CO_2 induced by a sudden change from gas mixtures 4 to 2, the deoxygenation due to the P_{O_2} decrease from 52 to 26 mmHg appears first and then the Bohr on-shift reaction recovers the S_{O_2} to the initial level at a slower rate. The opposite reaction, first oxygenation and second Bohr off-shift, was observed by flushing gas mixture 4 after regaining the equilibrium with gas mixture 2.

mmHg P_{O_2} to provide 0% and 100% S_{O_2} level, respectively.

The changes of S_{O_2} in the simple oxygenation and deoxygenation reactions are seen in Fig. 2a and b, respectively. The arrow in the recorded pattern indicates the time of the flush of a gas mixture. Precise analysis with a high speed response oscillograph showed that the half-time was about 0.4 s for both cases. The changes of S_{O_2} in the Bohr on- and off-shifts are shown in Fig. 3a and b, respectively. The S_{O_2} change was a simple exponential in every case. The rates of the Bohr shift reaction, however, were slower than those of the O_2 reactions shown in Fig. 2; the half-time was about 0.9 s for both shifts. Then, the cooperative kinetic reactions of O_2 and Bohr shift were performed in such a manner that the P_{O_2} around RBCs was varied together with the P_{CO_2} so as to make equal the S_{O_2} before and after the reaction (Fig. 4). If the O_2 affinity were to shift very rapidly as the P_{CO_2} was changed, the S_{O_2} change should not occur under such conditions that the P_{O_2} outside RBC was varied at the same rate as P_{CO_2} and as much as the O_2 backpressure. The S_{O_2} change appears as shown in Fig. 4, however, that is, the O_2 reactions appear first, and change the S_{O_2} , and the Bohr shift reaction follows to return the S_{O_2} level to its initial state. In another experiment, we found that the Bohr shift did not occur when activity of carbonic anhydrase was inhibited by acetazolamide. Additionally, the CO_2 diffusion into RBC is estimated to be 20 times as fast as the O_2 diffusion. Therefore, the rate of the O_2 affinity change due to the CO_2 may be retarded by the rate at which P_{CO_2} and pH come into equilibrium or the reaction rate of H^+ and haemoglobin. As a result, the Bohr shift might become slower than the oxygenation and deoxygenation reactions. At any rate, it seems certain that the Bohr effect is noticeably small in such a short time as the 1 s or so of the transit time through the gas exchange area.

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Correlation between biological potency and biodegradation of a somatostatin analogue

ONE approach to the problem of producing peptide hormones of high activity for clinical application is to synthesise analogues which are less rapidly inactivated¹. In the case of luliberin, it was found that an analogue with an inserted D-amino acid and a blocked C terminal end was 80 times more active than the native peptide *in vivo*. This correlated well with the decreased lysis of the analogue by neutral endopeptidases². There is at present considerable interest in utilising somatostatin clinically. This use is drastically limited by its very short half life of less than 5 min (ref. 3). Somatostatin inhibits the release of growth hormone, thyrotropin and prolactin from the pituitary, of glucagon and insulin from the pancreas, and also of gastrin³⁻⁵. As a consequence there is considerable incentive to prepare longer acting agonists for use in diabetes, and for treatment of developmental disorders.

In previous studies we established that two mechanisms are involved in the breakdown of somatostatin in rat brain: release of N-terminal Ala and Gly by an aminopeptidase, and cleavage at an internal site involving the aromatic residues Trp and Phe (Phe-Phe, Trp-Lys, Thr-Phe) (ref. 6; Fig. 1). Based on the sequential release of amino acids with time, the -Trp⁸-Lys⁹ bond seemed to be the most vulnerable. Early studies showed that aminopeptidase action is limited, and that carboxypeptidase activity is not a significant factor in breakdown. The only analogues available for the earlier study were N-blocked, such as N-acetyl-des-Ala¹-Gly²-somatostatin, but biological tests in man and animals have been disappointing^{7,8}. This may be explained by the failure to block endopeptidase activity at internal sites. In the present study, we have used another analogue containing a D-Trp in position 8 which is known to have an improved biological potency⁹. It is thus of considerable interest to determine whether the increased activity arises because of blocking an endopeptidase at the critical Trp⁸-Lys⁹ peptide bond (Fig. 1).

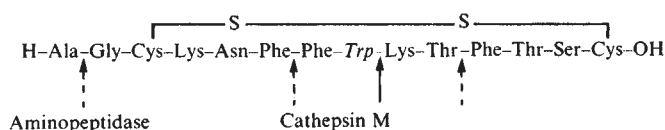


Fig. 1 Proposed primary ($\uparrow$) and secondary ($\downarrow$) sites for cleavage of cyclic somatostatin by a brain neutral endopeptidase (cathepsin M). Replacement of Trp in position 8 by D-Trp blocks cleavage of the D-Trp-Lys bond by the endopeptidase.

For studies on breakdown of (D-Trp⁸)-somatostatin in blood, and in rat brain homogenates, the strategy adopted was the same as that applied to luliberin, substance P, and kinin-9 (refs 2, 10 and 11). The method is simple in principle and is based on the timed release of amino acid constituents measured with the aid of an autoanalyser as described previously¹². In a crude mixture of enzymes some cleavage sites are rate limiting and the order of amino acid release provides information on the peptide bonds involved. This approach has been validated by the use of analogues with blocked cleavage sites, by the isolation of some of the intermediate peptide products and by the use of suitable bioassay procedures^{2,13,14}. This method is particularly useful when the nature of the inactivating enzyme is unknown, since it does not require preliminary purification of the enzyme nor the isolation of complex intermediate breakdown products.

Results for the critical residues adjacent to the sensitive bonds

Table 1 Cleavage of somatostatin and the D-Trp⁸ analogue by rat brain homogenate and rat serum

Substrate	Time (h)	Ala ^{1*}	Gly ²	nmol Lys ^{4,9}	% Trp ⁸	D-Trp ⁸	Phe ^{6,7,11}
Brain							
Somatostatin	4	17	37	45	40	—	37
D-Trp ⁸ -somatostatin	1	0	0	0	0	—	17
	4	24	26	0	—	0	28
Serum							
Somatostatin	4	44	50	58	60	—	46
D-Trp ⁸ -somatostatin	4	50	60	Trace	—	Trace	0

*Numbers refer to the position of residues adjacent to bonds sensitive to proteolytic cleavage in Fig. 1.

Trace, < 5 %.

Incubation mixture consisted of 50 nmol somatostatin or its D-Trp analogue in 0.2 ml Tris-HCl 10 mM, pH 7.6, containing 0.1 mM Cleland's reagent to which was added 0.2 ml 10% rat brain homogenate or 0.2 ml rat serum. Incubations were for 1–4 h at 37 °C and the reaction terminated by the addition of an equal volume of 6% w/v sulphosalicylic acid. Breakdown products in the supernatant fraction were assayed with the aid of an autoanalyser as described previously¹².

Potency *in vitro* (release of immunoassayable growth hormone) was 848 (confidence limits of 95%, 518–1416) compared with somatostatin at 100; *in vivo* (release of glucagon and insulin) was 821 (368–2195) compared with somatostatin at 100. Data provided by J. Rivier, Salk Institute, San Diego, California.

for the aminopeptidase and endopeptidase show a complete blockage of D-Trp⁸ and Lys⁹ release for the analogue when incubated with brain homogenate and serum (Table 1). In rat brain, the release of these two residues was reduced from 40–45 nmol % to zero, and release of Phe was reduced from 37 to 28 nmol %. In terms of the aminopeptidase action, release of Gly was reduced from 37 to 26 nmol %. With rat serum, the reduction in release of Phe was even more striking since no Phe was detected even after 4 h at 37 °C. In terms of biological activity, Rivier and his associates have shown that there is a six- to eightfold increase in the ability to inhibit the release of growth hormone *in vitro* and glucagon and insulin *in vivo*⁹.

These results are therefore consistent with the hypothesis that biological activity can be correlated with a change in biodegradation. It is therefore evident that in the case of brain the introduction of D-Trp in position 8 did not completely block the release of Phe (in positions 6, 7 or 11); this may account for an increase in potency but not necessarily for a longer duration of action. It would be necessary to block all internal sites to ensure a completely stable peptide in terms of neutral endopeptidase activity. There were some notable differences between cleavage of (D-Trp⁸)-somatostatin by brain and serum enzymes since the release of Phe was not observed with the latter. This may mean that (D-Trp⁸)-somatostatin is more stable during transport in serum but once it reaches its target site it can be inactivated at the secondary Phe⁶-Phe⁷ or Thr¹⁰-Phe¹¹ sites (Fig. 1). Since a larger proportion of the peptide would then be expected to reach the target site before destruction this could account for enhanced activity without a longer duration of action.

This explanation of the improved biological potency of the (D-Trp⁸)-somatostatin analogue does not exclude the possibility of an improved binding by the analogue to the relevant receptors. Studies on the binding of (D-Trp⁸)-somatostatin are unavailable, but in the case of other hormonal releasing factors there is often a remarkable correlation between increased activity and binding¹⁵. An improvement in receptor occupancy however does not necessarily ensure a longer duration of action and this may depend on a combination of factors, one of which is resistance to enzymic degradation.

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Covalently closed circular DNA molecules of low superhelix density as intermediate forms in plasmid replication

COVALENTLY closed circular (CCC) duplex extrachromosomal DNAs are widespread in biological systems¹. Because of their physical properties and small size, extra chromosomal circular DNAs are useful for studying the biology of genetic material. Their mechanism of replication is of particular interest and has been investigated extensively. Recent studies have shown that the replication of different types of circular DNA, such as plasmids of *Escherichia coli*, the DNA of polyoma and SV40 viruses, and mitochondrial DNA is basically similar^{2,3}. Non-replicating circular DNA is isolated from cells largely as covalently closed supercoiled molecules (form I DNA). Because of its topological constraints, CCC DNA has a restricted affinity for intercalating dyes such as ethidium bromide and propidium diiodide; its buoyant density in gradients of CsCl containing such dyes is consequently much greater than that of non-circular or nicked circular (form II) DNA species, which can unwind to accommodate maximal amounts of dye⁴. DNA molecules in the process of replication have been shown to have buoyant densities intermediate to form I and form II DNA (refs 5–12 and L. Katz, P. H. Williams, S. Sato, R. W. Leavitt, and D. R. Helinski, unpublished). In a previous study of replicative forms of a constructed recombinant plasmid (pSC134) of *E. coli*¹³, we observed material pulse labelled with radioactive thymidine that had a buoyant density in CsCl-ethidium bromide gradients even greater than that of form I DNA⁵. Here we report results which suggest that this material, termed “heavy replicative DNA” or hrDNA, is a covalently closed circular DNA species that has a reduced number of supercoils, and is an intermediate in the replicative process.

Figure 1 shows the kinetics of appearance and disappearance

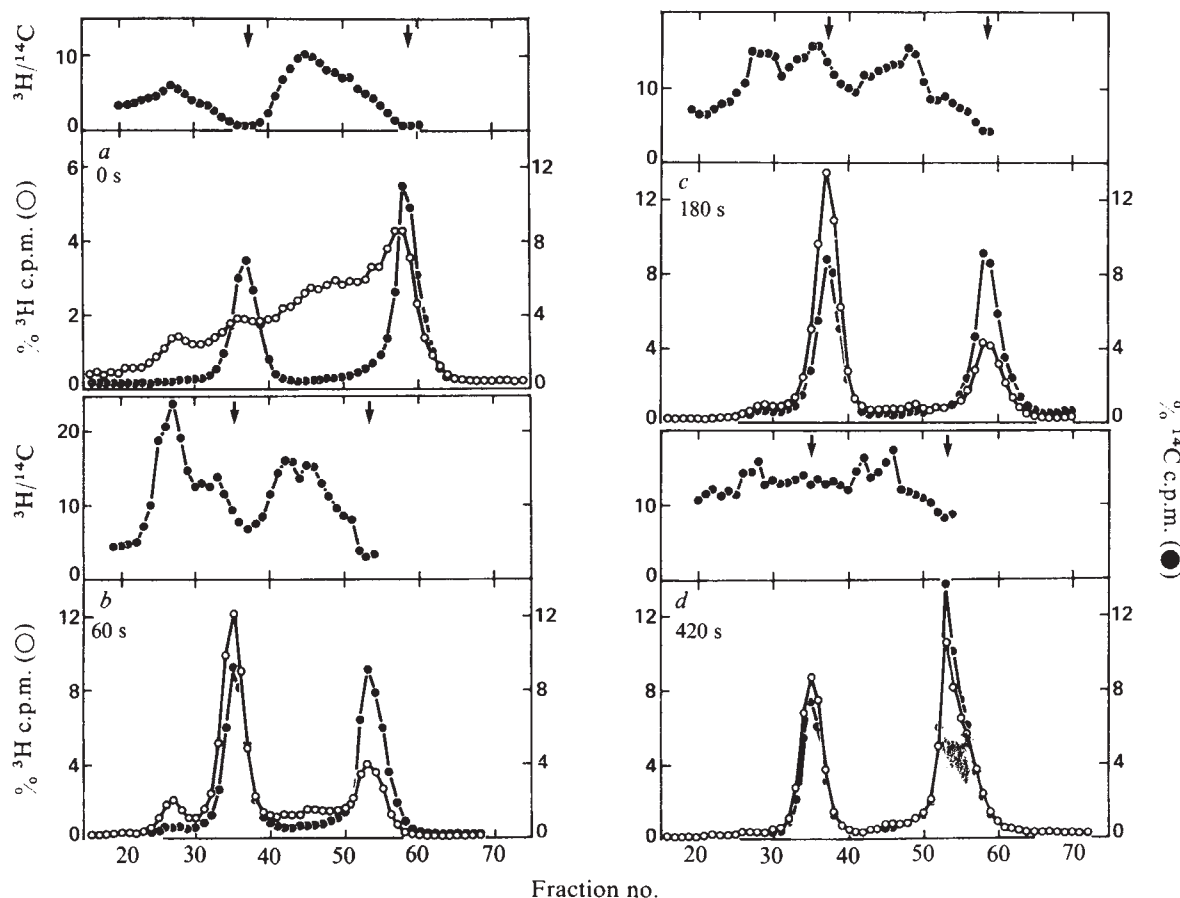


Fig. 1 Caesium chloride-propidium diiodide equilibrium centrifugation analysis of pulse-chase DNA from pSC101-carrying bacteria. 100 ml cultures of *E. coli* K12 CR34 Nal^r (pSC101)^{5,32} in M9 medium containing 0.5% casamino acids, 10 $\mu\text{g ml}^{-1}$ thiamine, 3 $\mu\text{g ml}^{-1}$ thymidine and 0.1 $\mu\text{Ci ml}^{-1}$ ^{14}C -thymidine were grown with aeration at 37 °C to an A_{590} of 0.4, resuspended in fresh, prewarmed medium without thymidine, and incubated for 30 min at 37 °C. The thymidine-starved cultures were cooled to 20 °C, pulse-labelled for 20 s at this temperature by addition of thymidine to 0.1 $\mu\text{g ml}^{-1}$ and ^3H -thymidine to 10 $\mu\text{Ci ml}^{-1}$ and then chased for various periods of time, (0, 60, 180, 420 s; panels a, b, c, d, respectively) by addition of non-radioactive thymidine to 500 $\mu\text{g ml}^{-1}$. Pulse-chases were terminated by pouring the cultures into 1,000-ml flasks which were precooled in dry-ice-isopropanol baths and which contained equal volumes of ice-KCN (final concentration 20 mM). Bacteria were washed twice

with 100 ml volumes of ice-cold TE buffer (10 mM Tris-HCl, pH 8.0–1 mM EDTA) containing 20 mM KCN, lysed with Triton X-100 and “cleared lysates” prepared⁵. The cleared lysates (8.5 ml volumes) were mixed with CsCl (8.0 g) and propidium diiodide (280 $\mu\text{g ml}^{-1}$) and centrifuged at 36,000 r.p.m. for 60 h at 20 °C in a Beckman 50 Ti rotor. Fractions were collected from the bottom of centrifuge tubes and the cold trichloroacetic acid, precipitable ^3H and ^{14}C radioactivity present in a 25- μl sample of each fraction was determined. In the figures, density increases from right to left. The upper part of each main panel shows the distribution of $^3\text{H}/^{14}\text{C}$ ratios. Arrows indicate the positions of non-replicating, covalently closed circular form I plasmid DNA (left) and nicked circular form II plasmid and non-circular DNA (right). Note that the magnitude of the ^3H scale for the 0 s chase is one-half that of the other chases.

of pulse-labelled hrDNA of the pSC101 plasmid. In this experiment, the total DNA of thymidine-requiring bacteria containing pSC101 was prelabelled with ^{14}C -thymidine and the cells were washed and then starved of thymidine for 30 min. Replicating DNA was pulse labelled by the addition of ^3H -thymidine, and the radioactive label was chased with cold thymidine for various periods of time. Extracts (“cleared lysates”) of bacteria were centrifuged to equilibrium in dye–CsCl gradients; in this instance we used the dye propidium diiodide to increase resolution of various DNA species¹⁴. Most of the DNA labelled during a 20-s pulse of ^3H -thymidine bands at a position between form I and form II DNA (Fig. 1a). Some of the pulse-labelled material is, however, seen to band in the gradient at a buoyant density greater than that of form I DNA. This is hrDNA.

After a 60-s chase with unlabelled thymidine, much of the radioactivity incorporated into the various pulse-labelled DNA species is found at the position of form I DNA; however, although the amount of labelled material present in the intermediate density peak is diminished, the amount of heavy material is increased (Fig. 1b). Thus, formation of hrDNA correlates with

the appearance of pulse label in mature supercoiled DNA and with the disappearance of replicative intermediates of intermediate density. With chases lasting more than 60 s, the amount of heavy material progressively decreases until it has disappeared by 420 s (Fig. 1c and d). The observed kinetics of appearance and disappearance of pulse-labelled heavy material suggest that it represents a plasmid replicative intermediate; it appears transiently during plasmid replication, and is rapidly converted into form I DNA during a chase with unlabelled thymidine.

hrDNA is also heterogeneous in buoyant density (Fig. 1) and seems to contain two major components that band 9 and 4 fractions, respectively, ahead of form I DNA in conditions which separate form I and form II DNA by 19 fractions (Fig. 1b). During the chase period, the amount of the heavier component of hrDNA decreases from 7% of the total ^3H -c.p.m. pulse label (60-s chase) to 2% (180-s chase) to 1% (420-s chase), but this component does not change position in the gradient. In contrast, during the same period, the lighter component of hrDNA moves progressively from a position four fractions ahead of form I DNA to a position of equal density to this DNA.

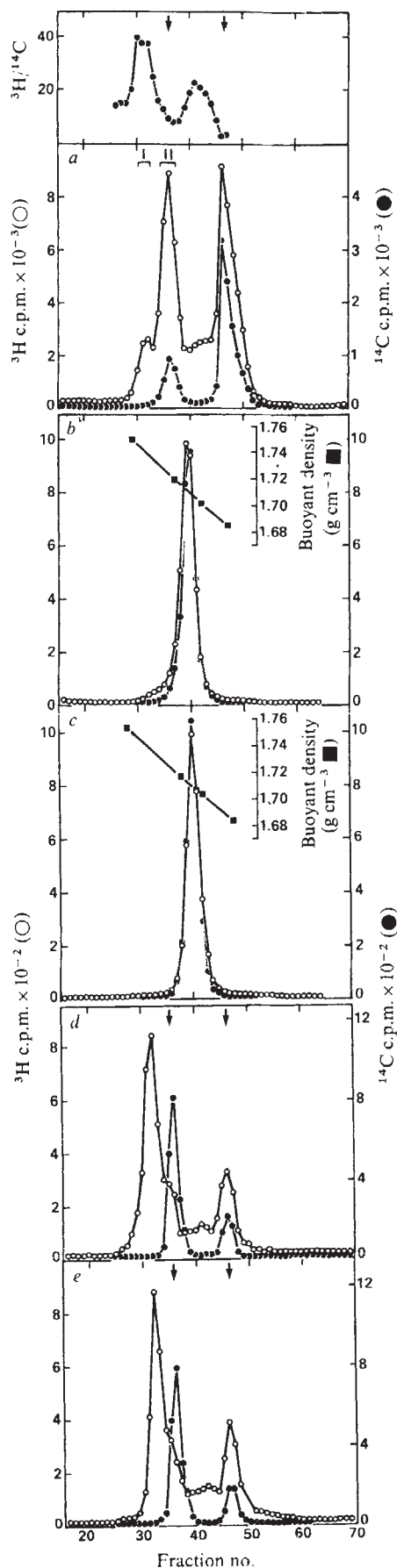
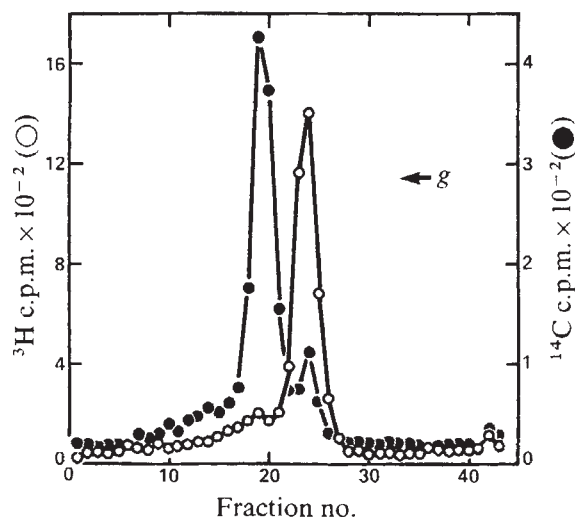


Fig. 2 Ribonuclease A digestion of heavy replicative pSC101 DNA. A 250-ml culture of *E. coli* K12 CR34 Nal^r (pSC101) was pulse labelled with ^3H -thymidine ($20 \mu\text{Ci ml}^{-1}$) and chased for 60 s with cold thymidine, as described for Fig. 1. A cleared lysate was prepared and 8 ml mixed with 8 g of CsCl plus 1 ml ethidium bromide (3 mg ml^{-1}) and was centrifuged to equilibrium ($36,000 \text{ r.p.m.}$ for 60 h at 20°C). Panel *a* shows the distribution of ^3H and ^{14}C radioactivity in this gradient. The dense replicative DNA obtained (pool I) was extracted twice with isopropanol equilibrated with TE buffer saturated with CsCl to remove ethidium bromide. It was subsequently dialysed for 3 h against 1,000 ml TE buffer (two changes), and samples treated (*c* and *e*) or not (*b* and *d*) with RNase A (Worthington) in low salt buffer, as described by Blair *et al.*¹⁹. After removal of the RNase by digestion with Pronase¹⁹, the DNA samples were mixed with ^{14}C -labelled reference pSC101 form I DNA and analysed by centrifugation to equilibrium in CsCl (*b* and *c*) or CsCl-ethidium bromide (*d* and *e*). The buoyant densities of selected fractions from the CsCl gradients were determined by measurement of their refractive indices. In *d* and *e* it can be seen that some nicking of CCC DNA had occurred after isolation but before RNase treatment. The proportion of nicked DNA was not increased by RNase treatment.

The position of hrDNA in dye-CsCl gradients could result from the presence of substantial amounts of RNA in the DNA molecules or from a decrease in their superhelix density¹⁶. To investigate the possible presence of RNA we prepared some of the heavier component of hr-pSC101 DNA (pool I of Fig. 2*a*) and analysed it by equilibrium centrifugation in CsCl gradients before or after treatment with RNase A at low salt concentration (Fig. 2*b* and *c*). An observed buoyant density shift in CsCl (0.001 g cm^{-3}) of ^3H -labelled hrDNA after RNase treatment to a position coincident with ^{14}C non-replicating pSC101 DNA showed the presence of an RNA component (calculated to represent about 1% of the DNA (ref. 16)) within hrDNA.

Centrifugation of RNase-treated heavy replicative material in CsCl-ethidium bromide gradients showed no detectable shift in buoyant density occurred following RNase treatment (Fig. 2*d* and *e*), indicating that the RNA shown to be present in hrDNA does not account for its banding properties in dye-CsCl gradients. Moreover, these data indicate that the RNA component of hrDNA is not covalently linked to the CCC DNA

Fig. 3 Velocity sedimentation analysis of heavy replicative pSC101 DNA. A sample of the dense material from pool I of Fig. 2*a* was dialysed for 3 h against 1,000 ml TE containing 1 M NaCl (two changes), mixed with ^{14}C -labelled reference form I pSC101 DNA and centrifuged through a 5.2-ml 5–20% neutral sucrose gradient at $40,000 \text{ r.p.m.}$ for 180 min at 20°C in a Beckman SW50.1 rotor. Fractions were collected on filters which were subsequently dried, washed with cold trichloroacetic acid and counted in a liquid scintillation counter.



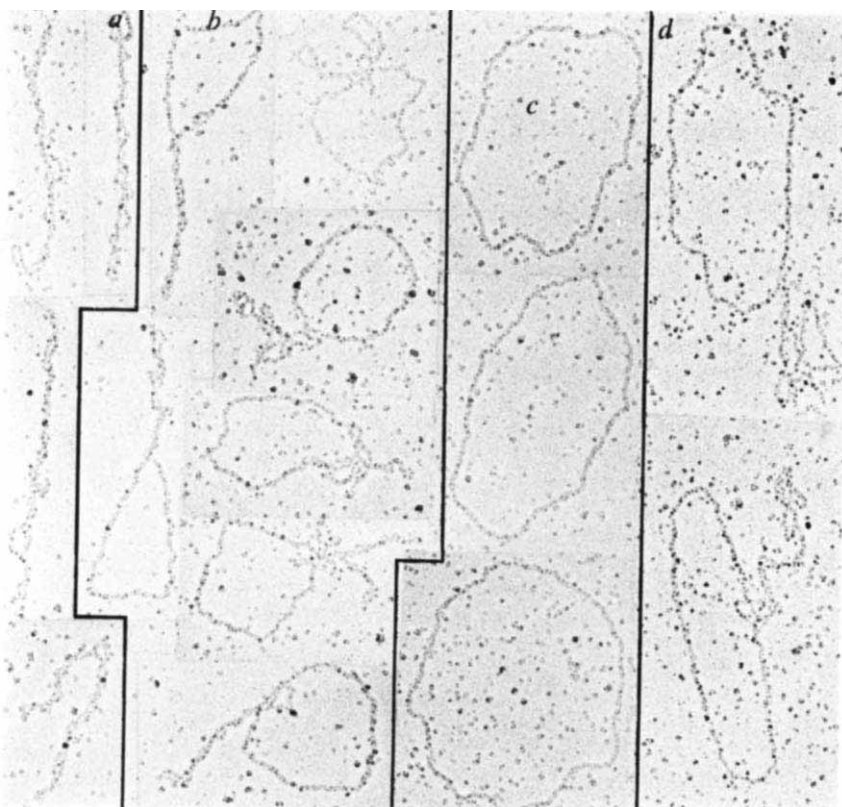


Fig. 4 Electron microscopy of heavy replicative pSC101 DNA. A sample of pool I from Fig. 2a was treated with isopropanol to remove ethidium bromide, dialysed against TE buffer and mounted for electron microscopy by means of the basic protein film method^{33,34}. Four main molecular forms of DNA were observed: highly supercoiled (form I) molecules (a); molecules with fewer superhelical turns, often with a partially open configuration (b); molecules with no supercoils (c); and catenated dimers consisting of one supercoiled monomer interlocked with a second open monomer (d; because these catenated dimers have buoyant densities equal to, or greater than, form I DNA, we conclude that the component of the catenate that lacks supercoils is nevertheless covalently closed). The relative number of each species present in this preparation are (for comparison, the relative number of similar forms present in the CCC DNA region of the same gradient, that is, pool II of Fig. 2a, are given in parentheses): a, 30 (134); b, 10 (1); c, 151 (3); d, 4 (4). The above photographs and numbers are derived from molecules present on several adjacent squares of a single grid. The reason for most molecules with a reduced superhelix density adopting a form with a terminal loop (b) is not known.

component, since RNase digestion does not produce nicking of this material (that is, conversion to a form II species). It remains to be determined whether the RNase-sensitive material associated with hrDNA is contaminating RNA or is RNA that has a plasmid-related function, such as the priming of DNA synthesis, as has been shown with ColE1 (refs 17–19).

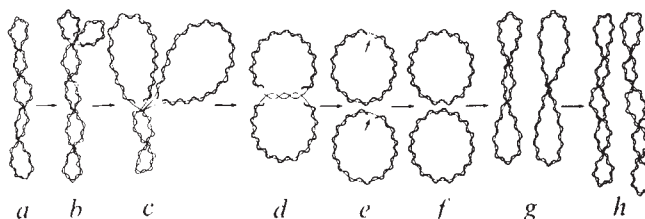
Analysis of hrDNA by neutral sucrose centrifugation also supports the view that it consists of CCC molecules with a reduced number of supercoils. Although its buoyant density characteristics in dye–CsCl gradients indicate that hrDNA consists of covalently closed DNA circles, the heavier component of hrDNA sediments with, or slightly ahead of, form II or nicked pSC101 DNA in 5–20% neutral sucrose gradients (Fig. 3), as expected for a monomeric DNA species lacking superhelical turns²⁰. The light components of hrDNA have greater sedimentation velocities (our unpublished data), consistent with an increasing number of twists as the DNA species approaches the buoyant density and superhelix density of form I DNA (ref. 20).

Because one might be able to demonstrate directly the postulated paucity of supercoils in the heavy material, we examined hrDNA (pool I of Fig. 2a) in the electron microscope and compared it with non-replicating form I DNA (pool II of Fig. 2a). Whereas most of the molecules from the form I region of a CsCl–ethidium bromide gradient are highly supercoiled (134 of 142; Fig. 4a), most of those present in pool I hrDNA are completely untwisted (151 of 195; Fig. 4c) and have the same appearance as nicked pSC101 circular monomers. Furthermore, some molecules that have a reduced superhelix density and seem to be partially open and partially supercoiled (10 of 195; Fig. 4b) are present in pool I DNA but are not present in significant numbers (1 of 142) in pool II DNA. Such molecules probably represent those present in the light component of hrDNA which is seen as a shoulder of form I DNA in dye–CsCl gradients (Figs 1 and 2). The types of molecules present in pool I DNA are thus consistent with the interpretation that hrDNA is composed of CCC molecules with reduced

superhelix densities. The absence of obvious replication “eyes” or forks in hrDNA is consistent with our inability to label this material with very brief (5 s) ³H-thymidine pulses in the absence of a chase (our unpublished data) and suggests an absence of nascent DNA. Thus, although these data do not exclude a role for hrDNA in the initiation of replication, they do support the results of our pulse-chase experiments which favour the alternative possibility that hrDNA represents one of the terminal stages of replication. It remains to be determined whether hrDNA is an obligate component of plasmid replication.

Earlier investigations^{2,3} have shown that replication is initiated at a specific sequence on the supercoiled (form I) DNA molecule (Fig. 5a), the replication origin, and proceeds either

Fig. 5 Schematic diagram of structural forms in the replication of circular DNA, indicating the proposed place of hrDNA in the cycle. Supercoiled CCC form I DNA (a) is progressively unwound during progeny strand synthesis (b, c) (ref. 9). Separation of the parental DNA strands at a time before completion of progeny strand synthesis (d, e) generates two daughter molecules with gaps (arrows) in the newly-synthesised DNA strands²¹. These gaps are subsequently filled and the strands are ligated (f), thereby generating covalently closed circles lacking superhelical turns, that is, hrDNA. Maturation of the progeny circular molecules presumably involves the progressive introduction of negative superhelical turns (g) until the winding number typical of form I DNA (h) is reached.



unidirectionally or bidirectionally away from this site, causing the parental strands progressively to unwind and separate (Fig. 5b-d). When the parental strands have been unwound completely, the progeny molecules separate (Fig. 5e). Initially they contain a "nick" or "gap" in the newly synthesised strand²¹. These nicked or gapped form II progeny molecules subsequently become converted to a covalently closed supercoiled form I species (Fig. 5h). Presumably, supercoils could be introduced into progeny molecules either before sealing of the closed circular form, or subsequent to an initial closing of the molecule.

Our finding that a closed circular monomeric DNA form lacking supercoils is an intermediate in plasmid replication suggests that newly separated progeny DNA molecules may be sealed to the closed circular form before the introduction of superhelical turns (Fig. 5f). Maturation of progeny DNA presumably involves the supercoiling of this species, possibly by means of specific DNA binding proteins and a swivel enzyme^{22,23}. If this supercoiling step occurs by a series of nicking-closing reactions (that is, is quantal), molecules with superhelix densities intermediate between that of form I DNA and DNA lacking supercoils (that is, with properties similar to those of the molecules present in the lighter component of hrDNA) may be generated (Fig. 5g).

The occurrence of covalently closed circular DNA molecules lacking supercoils as intermediates in the replication of circular DNA seems to be widespread. hrDNA has been observed in various *E. coli* plasmids (K. Timmis and F. Cabello, unpublished experiments, Y. Kupersztock, personal communication, and Crosa *et al.*²⁴) including ColE1, mini F (pSC138) (ref. 24) mini R6-5 (pSC135) (ref. 24), pSC134, F, and RSF1040. It has also been observed during the replication of mitochondrial DNA^{26,27}. Also, completely replicated CCC progeny molecules of SV40 and polyoma DNA of similar appearance and lacking superhelical turns accumulate in cells treated with protein synthesis inhibitors²⁸⁻³¹, suggesting that such molecules may also be intermediates in the replication of these viruses.

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Covalently closed circular DNA molecules deficient in superhelical density as intermediates in plasmid life cycle

WE have described¹ the replication of the conjugative R plasmid, RSF1040, a deletion mutant of R6K (ref. 2). RSF1040 possesses two distinct origins and replication generally proceeds from one or the other in an asymmetrically bidirectional mode to a unique terminus¹, although in several instances molecules with two simultaneous initiations were observed². During the course of examining the kinetic behaviour of replicating RSF1040 DNA (ref. 1), we observed ³H-thymidine pulse-labelled material that had a greater buoyant density in CsCl-ethidium bromide gradients than did mature covalently closed circular (form I) plasmid DNA. The nature of this material ('heavy DNA') and its relationship to the replication cycle are the subjects of this report.

Our previous study¹ has shown that only a small fraction of the DNA labelled during a short pulse (≤ 35 s) of ³H-thymidine banded in the region with higher buoyant density than form I DNA. We were unable to find any 'heavy DNA' in 5-10-s pulses, whereas longer pulses (> 35 s), always showed an appreciable amount of this material. Figure 1 shows the kinetics of DNA pulse-labelled for 50 s and its subsequent fate after a chase of unlabelled thymine plus thymidine as revealed by CsCl-ethidium bromide centrifugation of cleared lysates. Most of the DNA labelled during the 50-s pulse banded at a position intermediate between the ¹⁴C-prelabelled forms I and II (open circular) DNA (Fig. 1a). Approximately 9% of the pulse-labelled material, however, banded heterogeneously in two peaks at buoyant densities significantly greater than that of form I DNA. After a 60-s chase (Fig. 1b) the fraction of DNA occupying an intermediate density between forms I and II moved to lower density regions (that is, had replicated to a greater extent¹). Concomitantly the fraction of DNA banding in the form I region had increased to about 15% of the total replicating DNA and now occupied a position closer to, but still perceptibly more dense than, form I DNA. Subsequently, after a 180-s chase (Fig. 1c) virtually all the pulse-labelled DNA had disappeared from intermediate densities and was found in the form II as well as the form I positions of the gradient. Note, however, that although the fraction of ³H-thymidine-labelled DNA banding in the form I region had increased substantially, it still exhibited components slightly denser than mature non-replicating form I DNA.

Our observations that the 'heavy DNA' was not found when very short pulses were given prompted us to examine the fate of DNA labelled during a 25-s pulse and during the subsequent chase. Figure 2 concentrates solely on fractions adjacent to the form I region, including the regions of higher buoyant densities. These data indicate that some of the pulse-labelled material bands in the CsCl-ethidium bromide gradient at a buoyant density greater than form I DNA. As in the longer pulse of Fig. 1 this 'heavy' material, although heterogeneous, seems to contain two components banding at 7 and 3 fractions respectively, ahead of form I

DNA. After a 30-s chase, there is a net increase in material banding closer to the position of form I DNA, as well as an increase in the 'heavy DNA' sedimenting heterogeneously at about 7 fractions ahead of form I DNA. The subsequent chase samples showed a reduction in the 'heavy DNA' with

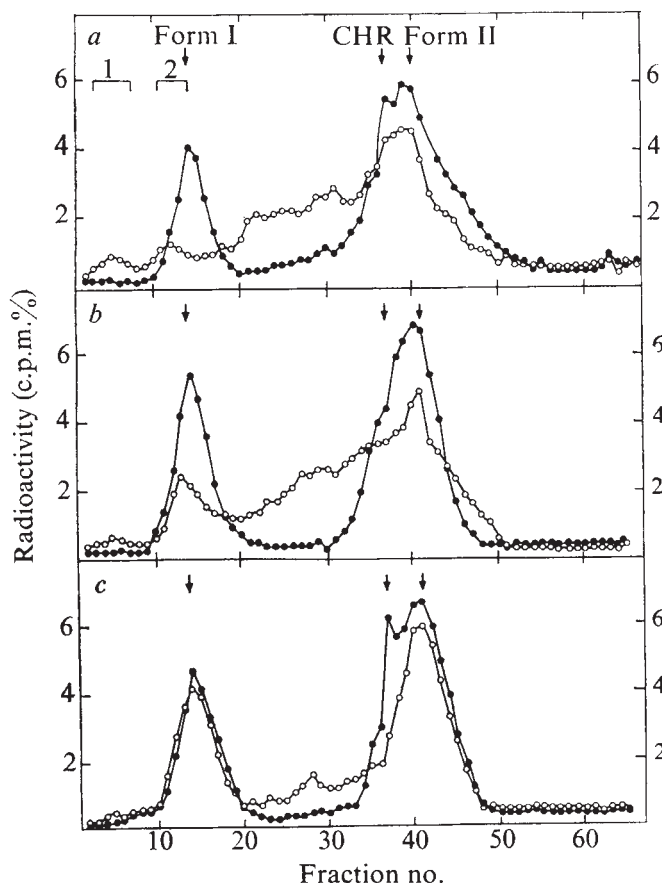


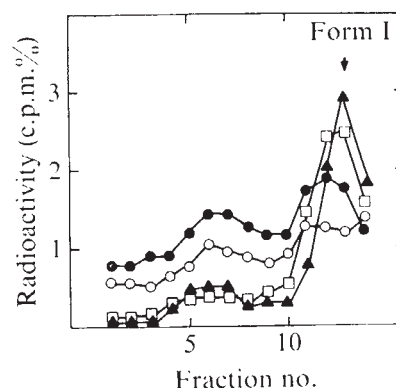
Fig. 1 CsCl-ethidium bromide density gradient analysis of a 'long' pulse-chase experiment. *Escherichia coli* K12 W1485-1 *F⁻thy⁻nal* containing RSF1040 (ampicillin resistant; *Ap^r*)² was used throughout this study. Cells were grown at 37°C with aeration in a 100-ml culture of M9 medium containing 0.5% glucose, 0.2% Casamino acids, 2 µg ml⁻¹ thiamine, 3 µg ml⁻¹ thymine and 0.6 µCi ml⁻¹ 2-¹⁴C-thymine, to a density of 2×10^8 cells per ml, collected by centrifugation at 25°C, resuspended into an equal volume of prewarmed medium lacking thymine and incubated at 37°C for 30 min (ref. 1). The culture was cooled to 25°C and pulse labelled at this temperature for 50 s with ³H-thymidine (20 µCi ml⁻¹; 0.05 µg ml⁻¹) and then chased for various periods of time (pulse 50 s, panel a; chases 60 and 180 s panels b and c, respectively), by addition of unlabelled thymidine (400 µg ml⁻¹) and thymine (20 µg ml⁻¹). Incorporation was stopped by addition of sodium azide (5×10^{-2} M, final concentration) and by freezing in a dry ice-ethanol bath. Cell suspensions were thawed and, after centrifugation, washed with cold TES buffer (50 mM Tris-HCl, 5 mM EDTA, 50 mM NaCl, pH 8) and resuspended to a density of 5×10^{10} cells per ml. The cells were lysed by the procedure of Clewell and Helinski¹⁸ using 0.05% Triton X-100, and 'cleared lysates' were prepared; 5.2 ml of such 'cleared lysates' were mixed with 4.9 g CsCl and 2 mg ethidium bromide and centrifuged at 40,000 r.p.m. for 64 h at 15°C in a 50 rotor of a Beckman L 3-50 ultracentrifuge. Five-drop fractions were collected from the bottom of centrifuge tubes into microtitre trays and 10-µl samples of each fraction were spotted on to 3MM Whatman filter paper circles, which were successively treated with cold TCA and ethanol and dried to determine the ³H (○) and ¹⁴C radioactivity (●) present in every fraction by counting in a liquid scintillation counter. In all panels the density increases from right to left. Arrows indicate the positions of covalently closed circular mature (form I) DNA and open circular or nicked (form II) DNA. The chromosome (CHR) bands two or three fractions ahead of plasmid form II DNA due to GC ratio differences².

concomitant increase in the regions closer to form I DNA. The data indicate that there is a dynamic shift in buoyant density of pulse-labelled DNA from that of the 'heavy DNA' to that which is indistinguishable from form I DNA. The observation that there is a transient appearance of the 'heavy DNA' during plasmid DNA replication and that the material is subsequently chased into form I DNA suggests that it represents a replicative intermediate form of the plasmid DNA.

The appearance of DNA with a buoyant density greater than that of non-replicating covalently closed circular DNA could be due to the presence of RNA complexed to replicating DNA or result from a difference in superhelical density³. To distinguish between these two possibilities, two pools (1 and 2, see Fig. 1a) were purified from a 50-s pulse-labelled lysate by centrifugation through a CsCl-ethidium bromide gradient. The purified DNA from each pool was treated with RNase at a low salt concentration⁴, and treated with pronase. Treated and untreated DNA were recentrifuged in CsCl gradients containing the dye propidium iodide in place of ethidium bromide, to increase the resolution of various DNA species⁵. Figure 3 shows that the banding of pools 1 (a and b) and 2 (d and e) DNA was unaffected by RNase-Pronase treatment. The small amount of form II DNA present was similar in both treated and untreated DNA samples, and presumably reflected nicking which occurred during experimental manipulation. Note, however, that centrifugation of both pools in neutral CsCl density gradients did show a very small, but reproducible, increase in buoyant density (0.001 ± 0.0001 g cm⁻³, average of 6 experiments) over that of ¹⁴C-labelled non-replicating form I DNA, which disappeared after RNase-Pronase treatment (our unpublished results). These results indicate the presence of RNA in 'heavy' replicating DNA. This RNA is not covalently linked to the closed circular DNA component since the RNase treatment does not produce nicking of this material. Whether this RNA is of functional significance remains to be shown, but certainly its presence was insufficient to account for the banding behaviour of pools 1 and 2 DNA in CsCl gradients containing intercalating dyes. We concluded, therefore, that the differences in band position arose from differences in superhelical density between pool 1, pool 2 and non-replicating form I DNA.

Treatment of pools 1 and 2 with the untwisting enzyme of rat cells (B. McConaughy and J. Champoux, unpublished) which untwists superhelical DNA to a covalently closed circular DNA species with no superhelix turns (relaxed or zero turns DNA), confirmed the view that these pools consisted primarily of molecular species containing a

Fig. 2 CsCl-ethidium bromide density gradient analysis of a 'short' pulse-chase experiment. Conditions were as described in Fig. 1, except that cells were pulse labelled with ³H-thymidine for 25 s and subsequently chased with unlabelled thymidine plus thymine; samples were withdrawn after various periods of time. The figure concentrates solely on the form I region and those of the region of higher buoyant densities. ○, 25-s pulse; ●, 30-s chase; □, 60-s chase; ▲, 180-s chase.



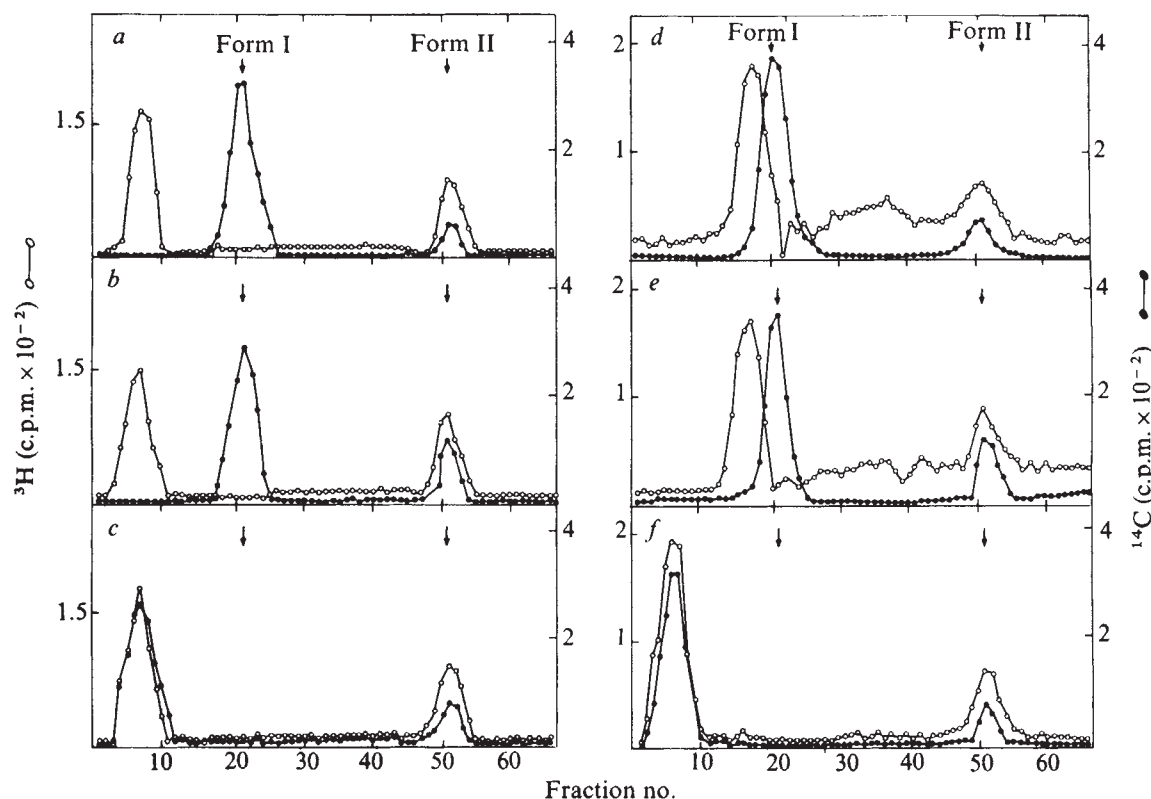


Fig. 3 RNase A and untwisting enzyme treatment of purified 'heavy' replicating RSF1040 plasmid DNA. A 400-ml culture of *E. coli* K12 W1485-1 (RSF1040) was pulse labelled with ^3H -thymidine ($20 \mu\text{Ci ml}^{-1}$; $0.05 \mu\text{g ml}^{-1}$) for 50 s. All subsequent manipulations were as described in Fig. 1. Centrifugation of the cleared lysate obtained in a CsCl-ethidium bromide density gradient gave a profile essentially similar to that of Fig. 1a. Pools 1 and 2 of 'heavy' replicating DNA were extracted separately with isopropanol (six times) to remove the ethidium bromide, and dialysed against 16 mM Tris-HCl, 5 mM EDTA, 16 mM NaCl, pH 8. RNase treatment consisted of the incubation of 50 μl pool 1 (or pool 2) DNA mixed with 0.1–0.5 μg ^{14}C -labelled form I RSF1040 DNA plus 1 mg ml^{-1} RNase A (Worthington) at 37°C for 60 min (ref. 14). The RNase was subsequently removed by incubation with self-digested (45 min at 37°C) Pronase (1.25 mg ml^{-1}). Untwisting enzyme treatment was carried out by incubation of 50 μl pool 1 (or pool 2) mixed with 0.1–0.5 μg ^{14}C -labelled form I RSF1040 DNA and sufficient enzyme to completely untwist 15.0 μg DNA in 30 min at 37°C in the standard assay conditions (B. McConaughy and J. Champoux, unpublished). After incubation the reaction was terminated by dilution with 0.01 M Tris-HCl and the addition of CsCl. Untreated controls consisting of the reaction mixtures without the enzyme treatments were also incubated for each of the reaction mixtures. All samples were then analysed by centrifugation in a CsCl-propidium iodide density gradient (4.8 g CsCl, 2 mg propidium iodide (Calbiochem) and 5 ml TES buffer). Centrifugation was at 40,000 r.p.m. at 15°C for 64 h in a 50 rotor. Fractions were collected directly on to 3MM Whatman filter circles and treated and counted as described in Fig. 1. ^3H -labelled pool 1 plus ^{14}C -labelled form I DNA: a, untreated samples; b, RNase-Pronase treatment; c, untwisting enzyme treatment. ^3H -labelled pool 2 plus ^{14}C -labelled form I DNA: d, untreated sample; e, RNase-Pronase treatment; f, untwisting enzyme treatment.

reduced superhelix density. A sample of pool 1 together with added ^{14}C -labelled form I DNA was treated with untwisting enzyme and banded in a CsCl-propidium iodide density gradient (Fig. 3c). The ^{14}C -labelled form I DNA which, after the untwisting enzyme treatment was converted to a form with zero superhelix turns, now banded at a buoyant density identical to that of pool 1 DNA which was unaffected by the treatment (compare Fig. 3c with the untreated control in Fig. 3a). The conclusion is that 'heavy' pulse-labelled DNA from pool 1 consists of covalently closed circular DNA with no (or few) superhelix turns. Indeed, examination of pool 1 in the electron microscope showed only open ring forms, and this material sedimented slightly ahead of form II DNA in neutral sucrose gradients (data not shown) as expected for a covalently closed circular DNA of those topological characteristics. Treatment of material from pool 2 with untwisting enzyme, as expected, converted it to a molecular species with a density identical to that of pool 1 DNA or untwisting enzyme-treated form I DNA (Fig. 3f). Since the distance between the open and closed forms of pool 2 DNA was about 10% greater than the distance between forms I and II non-replicating plasmid DNA (Fig. 3d) one can calculate (refs 6 and 7, and our unpublished results) that the molecules of newly synthesised

plasmid DNA banding at the position of the fractions of pool 2, on average, have a deficiency in the superhelical density of about 20%, compared with non-replicating form I DNA. It is clear from Fig. 2, however, that covalently closed circular RSF1040 plasmid DNA molecules encompassing a whole range of superhelical densities (from zero to that of form I DNA) may be isolated depending on the time of sampling after the ^3H -thymidine pulse.

Pulse-labelled DNA banding at positions more dense than mature form I DNA has been found in all plasmid systems examined by us so far (in addition to RSF1040, we have determined its presence in replicating DNA of plasmids RSF1010, RSF1030, R6K and ColE1; our unpublished results) and has also been characterised in pulse-labelled pSC101 (ref. 8).

Our results with RSF1040 (ref. 1), and those reported for other covalently closed circular DNA systems as ColE1 (refs 9 and 10, and L. Katz, P. H. Williams, S. Sato, R. W. Leavitt and D. R. Helinski, unpublished), pSC134 (ref. 11), SV40 (refs 12 and 13), polyoma¹⁴ and mitochondrial DNA (ref. 15) indicated that DNA synthesis occurred on a covalently closed circular template. The parental strands of replicating molecules were continuously unwinding during the replication cycle, presumably by a nicking and closing

mechanism involving endonucleolytic cleavage or a DNA untwisting enzyme^{16,17}. It is assumed that the unwinding of parental strands proceeds until all of the topological turns are removed and the daughter molecules can then separate, with at least one of the progeny molecules containing a nick and possibly a gap. A consequence of this assumption is that the nicked (or gapped) progeny (form II DNA molecules) will be closed subsequently and converted to form I DNA molecules, the end product of replication. Our earlier data¹, as well as those of other groups^{9,12,14}, indicated that form II DNA was indeed an intermediate during the replication of a covalently closed DNA molecule. The data presented in this paper suggest an intermediate stage between form II and form I DNA as one of the terminal events of replication. Form II DNA is presumably sealed to a covalently closed species with no superhelical turns. These transient forms appearing as 'heavy' DNA are in turn modified by the progressive introduction of negative superhelical turns until the superhelical density of form I DNA is achieved. This maturation process is presumably carried out through a series of nicking and sealing events, or by a protein or protein system which can introduce superhelix turns. Whether this type of transition is an obligatory step for all replicating form II DNA molecules remains to be demonstrated.

Earlier studies have shown that intracellular SV40 viral and PM2 phage DNA have lower superhelix densities than corresponding mature viral DNA (refs 7 and 19). Moreover, replicating SV40 and polyoma DNA accumulate in cells treated with protein synthesis inhibitors as closed circular molecules with very few turns²⁰⁻²³. Covalently closed molecules with low superhelical density have also been described for replicating mitochondrial DNA (refs 24 and 25).

The mechanism whereby superhelix turns may be introduced as a terminal step into newly replicated DNA is not established, although the formation of closed circular DNA of low superhelical density seems to be a common event during the life cycle of plasmid and viral DNA and may also prove of significance in the replication of circular molecules in other biological systems.

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Role for small nuclear RNAs in "programming" chromosomal information?

ALTHOUGH small nuclear RNAs (snRNAs) have been studied extensively^{1,2}, almost nothing is known about how they function in cells. I present here the results of a detailed study of the behaviour of snRNAs during mitosis, which has uncovered what may be the first important clue as to their function. I suggest, from observations of the pattern of snRNA association with mitotic chromosomes, that snRNAs are involved in specifying which genes are transcribed. Between 4 and 10 electrophoretically distinct snRNAs are found in species from protozoa to mammals, ranging from 65 to 200 nucleotides long. The snRNAs, which generally comprise less than 0.5% of total cellular RNA, are largely confined to the nuclei during interphase; some, however, are probably present in the interphase cytoplasm—although at concentrations much below that in the nucleus³. Such molecular characteristics as base compositions, methylation patterns and nucleotide sequences have been determined for at least some snRNAs, but have provided no recognisable clues regarding function. No evidence is available that snRNAs are part of the cellular translation machinery, as is true for the more abundant transfer, ribosomal and messenger RNAs, and their metabolic stability argues against a function as primers in DNA replication⁴. It has been suggested that certain snRNAs are related to ribosomal RNA processing⁵, cell proliferation⁶ and gene expression—but no data in support of these views are definitive. The snRNAs have been shown to be released to the cytoplasm during division, then to return virtually intact to the post-division daughter nuclei^{6,7}.

By transplanting ³H-uridine nuclei between directly labelled and unlabelled amoebae (*Amoeba proteus*) two or more times, it is possible to rid the nucleus (and the final recipient cell) of virtually all radioactive RNAs except the snRNAs (ref. 3). Because they are thus the only radioactive molecules in the cell, this technique allows us to follow clearly the behaviour of snRNAs during all subsequent experimental manipulations.

When an interphase cell containing a nucleus serially transplanted in this way is examined autoradiographically, the radioactivity is found to be almost entirely (>95%) in the nucleus (Fig. 1). The radioactivity seems to be uniformly distributed through the nucleus but, in fact, careful electron microscopic examination⁸ shows that the concentration of ³H is slightly higher over chromatin than over nucleoplasm but that the bulk of radioactivity is, nevertheless, non-chromosomal.

When a cell like that in Fig. 1 enters mitosis, much of the label is lost from the nucleus as the nuclear envelope breaks down, the nucleoli disappear, and the chromosomes condense. At metaphase the radioactivity is uniformly distributed through the cytoplasm, and virtually no label is associated with the chromosomes (Fig. 2). Thus, regardless of what may be the case at earlier mitotic stages, when the chromosomes are maximally condensed, essentially no snRNAs are associated with them.

The most unexpected result is observed just a few minutes later. At about the middle of anaphase the snRNAs are

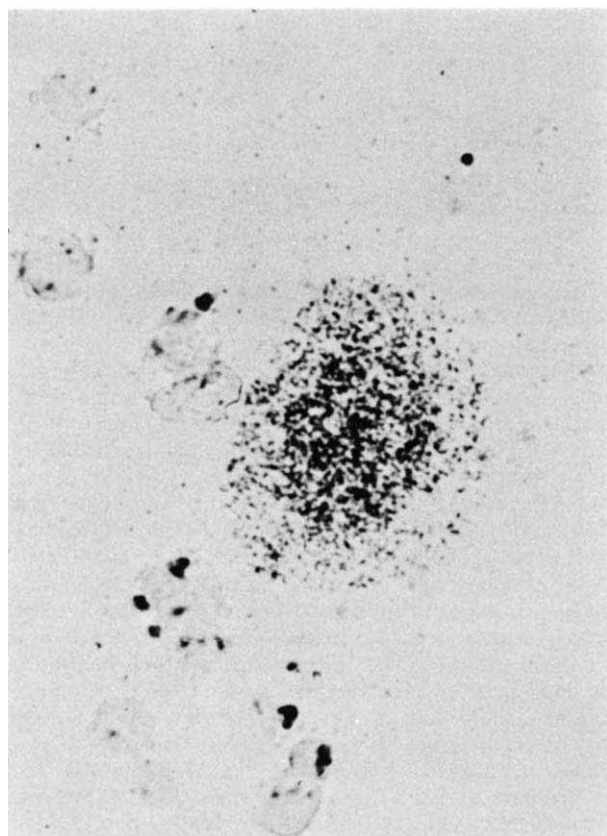


Fig. 1 Portion of an interphase cell ($\sim \times 700$ magnification) and its autoradiogram after the cell has been fixed and squashed with a coverslip containing a hanging drop of 45% acetic acid. The cell contains a nucleus transplanted¹⁵ through two unlabelled cytoplasts at 2-d intervals after the original donor cell had been labelled with ³H-uridine for 2 d and then fed unlabelled food for 3 d. Autoradiography was performed with Kodak's NTB-3. Note the virtual absence of label over the cytoplasm. The lesser amount of label around the nuclear periphery is attributable to the presence of a ring of nucleoli, which are more lightly labelled than the rest of the nucleus in these conditions.

found to be associated with the chromosomes to an enormous extent (Fig. 3), far more than had been the case during interphase. Thus, as the chromosomes begin to decondense, large numbers of sites apparently become available for the binding of snRNAs. As mitosis continues and the nuclear envelope reforms, the snRNAs begin to dissociate from the chromosomes but remain within the newly-formed nuclei. Some time during the first few hours of interphase, the distribution of snRNAs again becomes like that of the premitotic cell (Fig. 1); that is, although almost entirely nuclear, only a small proportion of snRNA is associated with chromatin.

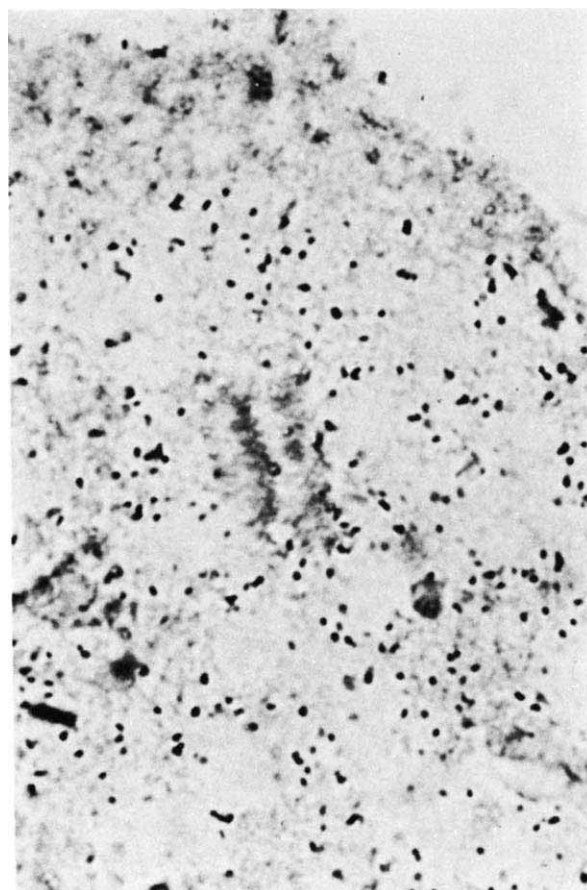
In preliminary electron microscopic studies (L. G., G. E. Wise, and C. Ko, unpublished), not only have these results been more clearly observed, but late prophase chromosomes have also been found to have a large amount of snRNAs associated with them. Thus, incompletely condensed (or decondensed) chromosomes apparently have a particular affinity for snRNAs.

From what is known of the behaviour of acidic nuclear proteins (ANPs) during mitosis and the role of 'quantal' mitoses in differentiation^{9,10} I propose a simple model of mitosis-associated rearrangements of chromosomal macromolecules, and the significance of those rearrangements—

borrowing from the ideas of Holtzer⁹ and Gurdon¹⁰. In amoebae, at least, the nuclear RNAs return from the cytoplasm in their entirety to the post-division nuclei within 15 min or less of the completion of telophase¹¹, whereas all of the ANPs (which are also released to the cytoplasm during the early stages of mitosis) take about 3 h (ref. 12). Thus, at least superficially, the snRNAs seem to be displaced from their chromosomal associations by the returning ANPs. Because we expect that only a small proportion of a cell's genes are being transcribed at any one time, the fact that only a small proportion of snRNAs remain associated with interphase chromatin suggests that snRNAs on chromosomes have a positive role in gene expression—presumably serving (perhaps by some specific base-pairing mechanism) to open up selected loci to transcriptive activity. (Such a model suggests that the rate of transcription ought to be unusually high immediately after mitosis, and examination of some data in connection with other experiments (L. Goldstein and C. Ko, unpublished) indicates that that may well be the case.) There is evidence that some small nuclear RNAs may stimulate transcription in specific ways¹³.

According to this model, specificity in locus selection need not reside in the snRNAs, but could well be a function of the ANPs, as has been frequently suggested (see, for example, ref. 14); the ANPs could act by displacing snRNAs from the selected chromosomal loci. That ANPs act thus in the 'programming' of chromosomes in the course of cell differentiation has been proposed by Holtzer⁹ and Gurdon¹⁰, who have noted that in spite of otherwise

Fig. 2 Cell ($\sim \times 1,500$), from the same population as cell in Fig. 1 but in mitosis at time of fixation, at the very beginning of anaphase (separating chromosome sets in middle of the picture). Cell sectioned at about 8 μ m and stained with Giemsa. Note virtual absence of autoradiographic grains over chromosomes.



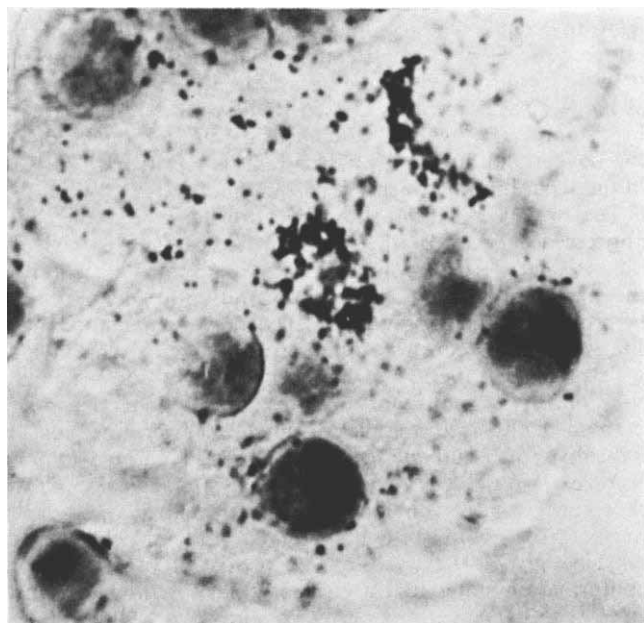


Fig. 3 As Fig. 2, except cell ($\sim \times 2,000$) is in mid-anaphase. (Round, dark-staining bodies are food vacuoles.) The stained chromosome sets are obscured by the high concentration of autoradiographic grains.

favourable conditions certain kinds of cells do not change their phenotype unless they undergo mitosis (called a 'quantal' mitosis) subsequent to the occurrence of the differentiation-inducing condition. Such an hypothesis seems to require that the primary event leading to differentiation is, for example, an environmentally-induced change in the cytoplasmic spectrum of certain latent ANPs that cannot immediately associate with chromosomes to affect transcriptive activity either because the nuclear envelope is a barrier or because ANPs already associated with the chromosomes cannot be displaced. When the affected cells enter into mitosis, however, the 'old' ANPs (and snRNAs) are discarded from the chromosomes, thus ostensibly enabling others from the cytoplasm to associate with the decondensing chromosomes from anaphase onward.

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Kinetics of triplet-triplet energy transfer and intramolecular distances in enzyme-inhibitor complexes

We wish to report an application of the relationship between the kinetics of triplet energy transfer and donor-acceptor geometries to biomolecular systems. Our study illustrates the usefulness of measurements of this type in estimating molecular distances and providing a measure of variability within enzyme-inhibitor complexes.

The sensitivity of radiationless transfer of triplet electronic excitation to distance¹ prompted its use for exploring the proximity of chromophores in nucleic acid² and protein complexes³, and triplet-triplet energy transfer has been demonstrated in various biomolecular systems in rigid solution⁴⁻⁷. In favourable cases the transfer has been quantitated from the quenching of the donor phosphorescence in terms of a transfer efficiency. Although it has been possible to establish an upper limit on the distance between donor and acceptor groups from 'active sphere' radii¹ for transfer in model systems, the lack of an experimentally determined distance dependence for the efficiency has limited the usefulness of triplet transfer as a measure of the separation between chromophores. Furthermore, although the transfer seems efficient when it proceeds at a rate which is more rapid than the normal triplet decay rate ($0.1-10^2 \text{ s}^{-1}$), studies with solutions^{8,9} and molecular crystals¹⁰ indicate that triplet transfer occurs at a rate orders of magnitude more rapid than this as molecules approach van der Waals' contact. The more rapid transfers corresponding to shorter intermolecular separations can be monitored if the transfer kinetics, rather than transfer efficiencies, are measured directly.

Rates of triplet-triplet transfer have been determined from the decay of the phosphorescence of the donor following laser-pulsed excitation^{11,12}. An exponential dependence of the transfer rate constant on distance of the form predicted by Dexter¹³ has been found and empirical parameters relating transfer with distance have been established for several donor-acceptor pairs^{11,12,14}. In our study the rates of energy transfer from a sulphonamide inhibitor to tryptophan residues in bovine and human B and C forms of carbonic anhydrase have been measured and translated into the corresponding distances.

When the energy donor (*m*-acetylbenzene sulphonamide)⁸ bound at the active site of all three carbonic anhydrases is excited selectively at 330 nm, the steady state phosphorescence of the sulphonamide is replaced by a sensitised tryptophan phosphorescence. The marked reduction, or quenching, of the donor emission indicates that the efficient transfer which had been demonstrated earlier for the bovine enzyme³ is also operative in both human forms of the enzyme. Efficient triplet transfer with the same inhibitor has also been observed (A. Maki, personal communication) during an optical detection of magnetic resonance (ODMR) study of these systems.

Although the steady state phosphorescence results establish that transfer is efficient in all three enzymes, our flash experiments reveal that the transfer rates differ significantly. The decay of the phosphorescence of the donor after an exciting pulse at 337 nm from a small N_2 laser is depicted in Fig. 1. Transfer occurs with a rate constant of $2.4 \times 10^4 \text{ s}^{-1}$ in the bovine enzyme; 10^4 s^{-1} for 70-80% of the transfer in the human C enzyme with the remainder occurring more rapidly; and at a rate which was too rapid for reliable detection ($> 10^6 \text{ s}^{-1}$) in our experiments with the human B form. Although rates of 10^4-10^5 s^{-1} are sufficiently large compared with the normal donor decay (half value = $3.3 \times 10^2 \text{ s}^{-1}$) to produce the efficient transfer observed, they are orders of magnitude less than would be anticipated for molecules in van der Waals contact.

Ignoring the potential contribution of the relative orientation

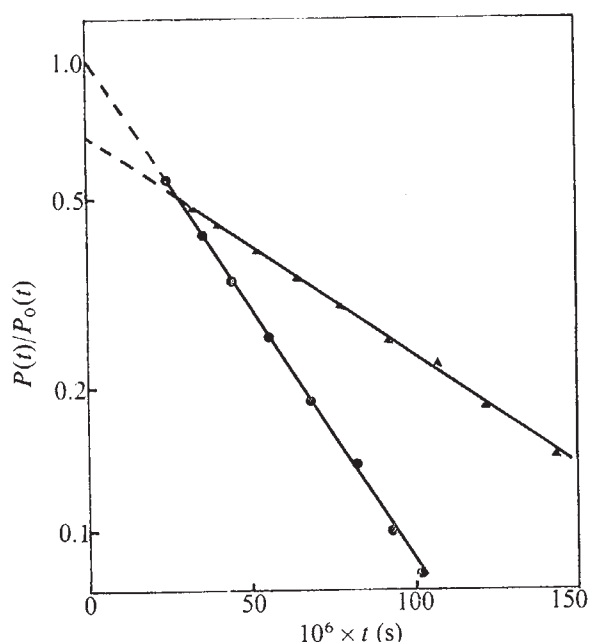


Fig. 1 Decay of the phosphorescence of *m*-acetylbenzene sulphonamide (donor) due to energy transfer to tryptophan residues in human carbonic anhydrase C (Δ) and bovine carbonic anhydrase (Sigma) ($\bullet$). The sulphonamide inhibitor was synthesised from *m*-acetylbenzene sulphonylfluoride (Aldrich) by addition of ammonia³. Kinetic measurements were made with solutions of native enzyme (1.2×10^{-3} M) plus inhibitor (1×10^{-3} M) in 1:1 ethylene glycol: phosphate buffer (0.1 M, pH 8.0) at 77 K. The sulphonamide inhibitor was excited selectively at 337 nm with a pulse (< 20 ns) from a molecular nitrogen laser¹⁶. The decay of the donor phosphorescence after pulsed excitation was monitored at 390 nm. The donor phosphorescence intensity in the absence, $P_0(t)$, and presence, $P(t)$, of energy transfer can be represented as

$$P_0(t) = P(0) \exp(-t/\tau_0) \\ \text{and } P(t) = P(0) \exp(-t/\tau_0) \sum_i \alpha_i \exp(-k_{Ti}t)$$

where τ_0 is the donor lifetime in the absence of transfer and α_i is the fraction of donor molecules which transfer with a rate constant k_{Ti} . The contribution to the decay from energy transfer was evaluated from

$$\ln P(t)/P_0(t) = \ln \left(\sum_i \alpha_i \exp(-k_{Ti}t) \right)$$

which for a single rate constant reduces to

$$\ln P(t)/P_0(t) = -k_{Ti}t$$

of the donor and acceptor to the transfer, we have translated the measured triplet-triplet energy transfer rate constants k_T into distances using the relationship

$$k_T = 8.3 \times 10^{15} \exp(-2.63r)$$

found for transfer between acetophenone and indole in solid solution¹⁴. This results in a distance or an average distance of 10.1 Å from the centre of the sulphonamide to that of one or more tryptophan residues in the bovine enzyme, 10.4 Å for the slowly transferring component in the human C enzyme and < 9.5 Å in the B form. The absolute distances must be considered as somewhat uncertain because the magnitude of the role of molecular orientations in the transfer process is not known. Using the coordinates (K. K. Kannan, personal communication) for the structure of the crystalline human C enzyme¹⁵, and with the assumption that our energy donor binds in a manner analogous to that of a very similar sulphonamide¹⁵, we have estimated the distances from the

inhibitor to the tryptophan residues in the enzyme. The two closest tryptophan residues lie at a distance (10.3 and 10.6 Å) from the centre of the sulphonamide which is similar to that estimated from our transfer rate measurements for the enzyme in rigid solution. More significant in this respect than the absolute measurements is the sensitivity of the transfer kinetics to distance. The twofold difference in rate constant found in the bovine and human C enzyme reflects a difference of 0.3 Å in the separation between the chromophores.

The presence of more than one transfer rate in the phosphorescence decay of the donor indicates the absence of a unique spatial relationship between donor and acceptor. In the enzyme-inhibitor complexes a variation in geometry could be produced either from the presence of more than one binding site within the "substrate binding cavity"¹⁵, or from a more widespread distribution of conformations for the proteins in solution. It is significant that in the sulphonamide-bovine carbonic anhydrase complex a single transfer rate constant indicative of a unique geometry for the enzyme-inhibitor complex is observed. A variation of > 0.2 Å in the donor-acceptor distance would have been detected in these experiments. Most inhibitor-containing enzyme molecules in the ethylene glycol-H₂O solvent must exist in a well defined conformation state. It will be interesting to compare transfer rates of this type with measurements in the crystalline state to ascertain whether the transfer rate constants reflect very similar geometries.

We feel that the sensitivity of kinetics of triplet energy transfer in exploring the variability within biomolecular structures and in detecting subtle changes in distance recommends the use of measurements of this type in the investigation of biomolecular conformations.

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Stereoselectivity of β irradiation of D,L-tryptophan in aqueous solution

A POSSIBLE connection between the molecular asymmetry of living matter and the intrinsic asymmetry of elementary particles¹ was first suggested by Vester². All the experiments based essentially on the non-conservation of parity in weak interactions^{3,4} can be classified as follows: (1) It is assumed that

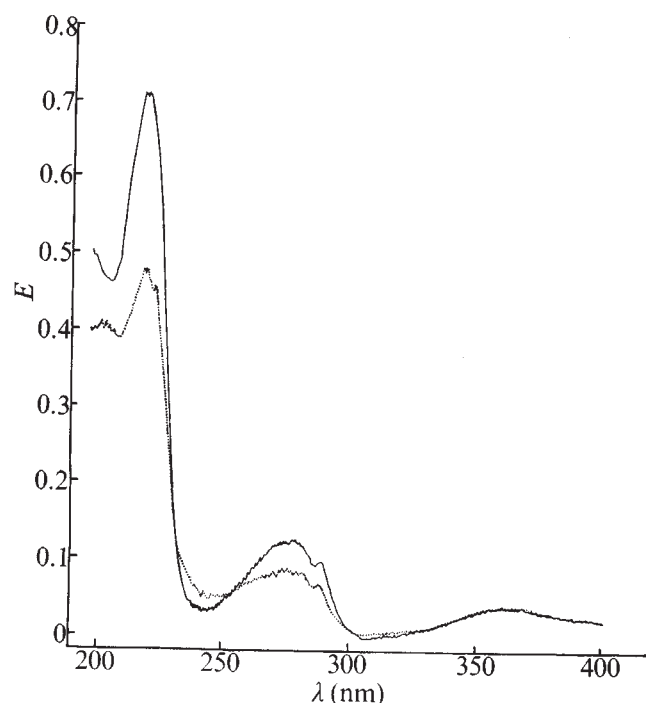


Fig. 1 UV-absorption spectrum for irradiated (.....) and non-irradiated (—) tryptophan samples ($c = 10^{-5} \text{ mol l}^{-1}$).

L- and D-enantiomers are not exact mirror images of each other but differ in their scalar physicochemical properties^{5,6}. Parity-violating forces originated by weak interactions could in principle give rise to different bond strengths in enantiomer molecules. If the recently discovered neutral weak currents turn out to be of vector and axialvector type—an opinion favoured by present neutrino experiments⁷⁻⁹—there is necessarily an

interference between parity-conserving electromagnetic interaction and parity-violating weak interaction; it might, however, be very small²³. The question whether a particular molecular quantum state does allow for the actual occurrence of a seizable parity-violating effect has, to our knowledge, not yet been settled. (2) It is supposed that the almost exclusive existence of one enantiomer in the biosphere is due to the asymmetric interaction of longitudinally polarised β rays with molecules (interaction either with racemic mixtures or with molecules undergoing reaction to form asymmetric compounds) during the early history of the earth. With this view it is believed that an induced optical activity is a result of the natural radiation from isotopes in the earth's crust^{4,10,11}. Experiments which aimed to generate optical activity in this manner are summarised in Table 1.

In an attempt to assess the validity of the latter hypothesis we investigated the irradiation of D,L-tryptophan with β rays from ^{32}P (mean energy of β rays: 0.57 MeV, degree of polarisation: 93.9%) in homogeneous solution (5 mg D,L-tryptophan in 100 ml twice-distilled water). Sixteen aliquots (2 ml) of this solution were filled into Pyrex ampoules. 5 mCi ^{32}P (as phosphate in 0.02 N HCl carrier-free in 0.2 ml) were added to eight of these ampoules; the remaining eight (controls) were diluted with 0.2 ml 0.02 N HCl. All sixteen ampoules were sealed and left at -25°C for 12 weeks. The energy absorbed during this period by the whole system would be $1.9 \times 10^{20} \text{ eV}$. After thawing and opening the ampoules aliquots of 2 ml were withdrawn and diluted with distilled water to 20 ml. Samples were transferred directly to a quartz cell to measure the ultraviolet absorption spectrum at 200–400 nm and to determine the optical activity at 220 nm.

The procedure for determining angles of rotation in the millidegree range has been described in detail elsewhere¹². Figure 1 shows that the overall decomposition of tryptophan by irradiation with ^{32}P after 12 weeks is about 33%. Figure 2 shows that the 'specific angle of rotation' at 220 nm is equal to $-3.6 \text{ millidegrees cm}^{-1} \times 10^{-5} \text{ mol l}^{-1}$ ($[\alpha] = -17,600$). Table 2 shows that the eight irradiated samples have a positive angle of rotation of $+0.7 \pm 0.4 \text{ millidegrees}$ (mean value of sixteen independent runs).

Table 1

Reference(s)	Investigated system	Irradiation source	Intensity	Time of irradiation	Analytical method	Result
15	Organic syntheses and decompositions involving free radicals and carbonium ions	β^- emitters: ^{32}P , $^{90}\text{Sr}/^{90}\text{Y}$, ^{52}Eu , ^{108}Ag , ^{110}Ag	30 mCi–1 Ci	5–2,900 min	α at Na–D line	No effect
17	Optically active and racemic substrates in solid state	β^- emitter ^{104}Rh and electrons from accelerator	20–200 Ci		α at Na–D line	No effect
16	L- and D-tyrosine in aqueous solution	β^- emitter $^{90}\text{Sr}/^{90}\text{Y}$	0.36 mCi	1.5 yr	Ultraviolet absorption spectrum	D-tyrosine more decomposed
18	^{14}C -labelled racemic norvaline, alanine, dopa, aspartic acid, methionine in solid state	β^- emitter ^{14}C , autoradiolysis		12–24 yr	ORD	No effect
19	Optically active and racemic norleucine, glycine, proline in solid and dissolved state	Bremsstrahlung from β^- emitter $^{90}\text{Sr}/^{90}\text{Y}$	61.7 kCi	1.34 yr	ORD between 250–630 nm and GC	No effect
20	L,D-phenylalanine, L,D-tyrosine, L, D-tryptophan	β^+ emitter ^{22}Na	0.8 μCi		Triplet positronium and annihilation time	D-isomers favour triplet state
21	D- and L-leucine	Left- and right-polarised electrons from accelerator			ORD, GC	Left-handed e^- destroy D-leucine preferentially and vice versa
22	D,L-alanine, D,L-octanol solid and dissolved	μ^+ particles from accelerator			Formation of muonium and subsequent chemical reaction	No effect

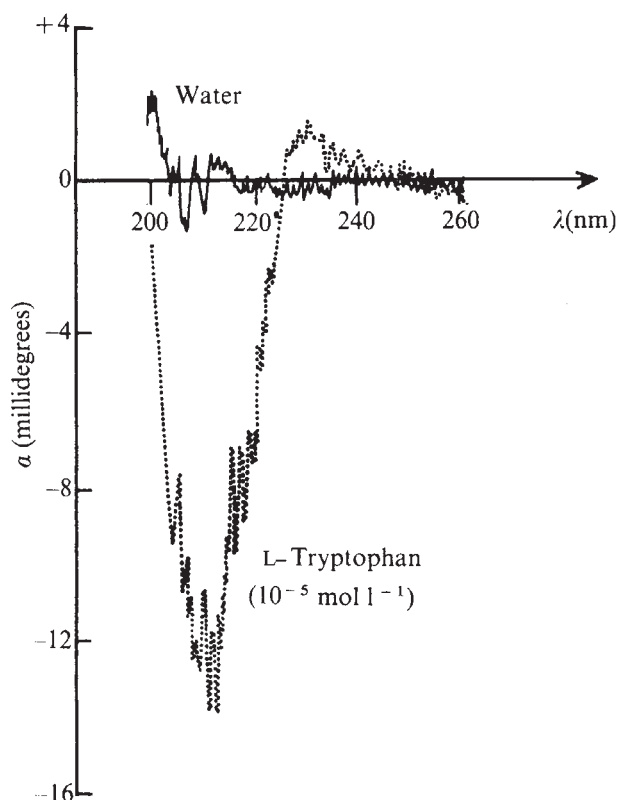


Fig. 2 Typical ORD of a diluted untreated sample of L-tryptophan in 0.002 N HCl ($c = 10^{-5} \text{ mol l}^{-1}$).

From the comparison of these values it seems that a preferential destruction of L-tryptophan has taken place, resulting in a relative enrichment of about 19% of the D-enantiomer. This is in qualitative agreement with previously reported experiments on the interaction of D- and L-tyrosine with β particles¹⁶. In our case, however, the L-enantiomer was decomposed to a greater extent than the D-enantiomer, whereas Garay¹⁶ found that D-tyrosine was preferentially destroyed. (Note that the CD of tryptophan and tyrosine in ultraviolet are of opposite sign.) It may be that the different structure of these amino acids is of importance in connection with these results. Our experiments were designed to meet the criticisms of Garay's early work in which enantiomers had been studied in separate batches (which

left uncertain the identity of the experimental conditions in the two cases); and the direct approach of irradiating a racemic mixture to produce optical activity had not been carried out.

Recently, two additional results have been obtained concerning the origin of chirality in nature, Kovacs and Garay¹³ reported a preferential influence of polarised β particles on the crystallisation of racemic compounds, which is difficult to understand in the light of existing classical theories. Furthermore, a particularly startling experiment has been reported by Merwitz¹⁴: Irradiation of solid L-, D- and D,L-phenylalanine with non-polarised γ particles had a discriminating effect on the decomposition of the target substance. Very large differences in the production of CO_2 from the main decarboxylation reaction were observed. This latter result cannot be interpreted on the basis of polarised particles as proposed in the original 'Ulbricht-Vester mechanism'^{4,10,11,15}. This result supports the above-mentioned view that there are differences in the scalar properties of enantiomers. As these energy differences are very small they may have been amplified in some kind of chain reaction.

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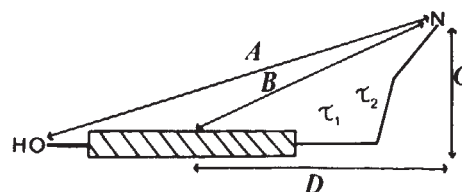
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Corrigendum

In the article "Structural and conformational relationships between the enkephalins and the opiates" by A. S. Hojn and J. R. Rodgers (*Nature*, **260**, 795; 1976) Fig. 1c is more correctly depicted as shown below and the mean value for the parameter D is 4.3 Å not 4.4 Å.



Mean $\alpha = +0.7 \pm 0.4$ millidegrees.

*Data are already corrected and calculated from the statistically weighted recorded¹² α for each sample and reference (zero) pure solvent.

matters arising

Solar activity on August 12, 1975

ATTENTION has been called to the unusual number and proportion of Type III-RS (reverse slope) solar radio bursts observed on August 12, 1975 (ref. 1). We believe a simple explanation of these observations is suggested by comparison with H α filtergram movies obtained at Big Bear Solar Observatory on that date. We observed a number of the radio bursts to coincide in time with a homologous set of flares at the preceding edge of Mount Wilson spot group 19598. This group was at $\sim 45^\circ$ W, thus our line of sight to the flares lay through the corona above the spot group. We suggest that the bursts were emitted as U bursts by an electron stream travelling from the flare site along a closed field line to the following part of the group. The emission at the plasma frequency on the preceding side of the field line could not propagate through the coronal density enhancement over the spot group to Earth. Only on the following side, where there was no intervening region of higher density, could the emitted radiation be observed. Therefore the bursts were seen only where the electrons were moving downward in the corona, producing reverse slope bursts. A detailed study of these events is in preparation.

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Oscillations in tropical ecosystems

BIGGER¹ has provided examples to show that population oscillations do in fact occur in the tropics. My purpose here is to show that his observations actually provide evidence in favour of the suggestion² that the linear connectivity of complex ecosystems is low, even though they contradict a deduction that was drawn from this.

There are essentially three types of oscillations which we might expect to observe in an ecosystem. Two of these

are produced by interactions within the system; these are Lotka-Volterra oscillations around a stable steady state and limit cycle oscillations around an unstable steady state. The third possibility, which was not taken into account by Saunders and Bazin, is forced oscillations, in which a system which would otherwise be in a stable steady state oscillates either in response to a cyclic environmental perturbation or else on account of some autonomous oscillation of one species.

When oscillations are caused by interactions, the periods are chiefly determined by the parameters of the system. In such a case we would not expect the periods to be almost all very nearly one or two years; this is much more likely to indicate that the oscillations arise from seasonal variation in the environment. Bigger's observations are thus not in conflict with the suggestion that the structure of tropical ecosystems is not such as to produce oscillations. Moreover, that the response of the system to a periodic perturbation is a stable oscillation with the same period is evidence that the system is overdamped; were this not the case we would expect there to be oscillations with different periods (determined by the parameters of the system and not, in general, equal to one generation length) superimposed on the annual cycle.

The evidence, then, still supports the contention that the linear connectivity of complex ecosystems is low and that it is this that accounts for their stability. It follows that it is not likely to be profitable to think of complex ecosystems as generalised Lotka-Volterra systems which can be adequately analysed in terms of the linear parts of their interactions. Indeed, if it is true that even the lynx cycle in Canada is a forced oscillation³, then the Lotka-Volterra equations themselves may be directly applicable almost nowhere in ecology, though they may remain useful for conceptual purposes.

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¹Bigger, M., *Nature*, **259**, 207-209 (1976).

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Evidence for polygenes

THOMPSON¹ has suggested that the generally held belief among quantitative geneticists that quantitative variation is determined by a large number of loci is an assumption which "unfortunately, some have come to think of . . . as an established fact". Fisher² considered in detail the hypothesis that some traits were determined by a large number of Mendelian factors. The assumptions made in that paper are the consequence of this hypothesis. He showed, as he says³, "that the heredity behaviour to be observed does not become more complex" as the number of factors is increased. The evidence available, so far, favours this hypothesis.

Thompson¹, however, argues that "there is no compelling reason to think that the total number of loci affecting a quantitative character is exceptionally large". He believes that a "relatively small" number of genes could account for the genetic variance of a quantitative character; however, there are a number of published cases where a large number of loci is indicated.

Consider 3 loci, each with two equally frequent alleles, no dominance and equal effects. With environmental effects the distribution of the trait determined by these loci will be approximately normal. If 10% of the population are selected as parents, then after two generations the parental population and their progeny will be genetically homogeneous. The larger the number of segregating loci the larger will be the number of generations required to obtain a genetically homogeneous population. This will be true in the presence of polymorphism, as well.

Falconer³, discussing the results of four selection experiments, says "the response continues for about 20 to 30 generations and the total range is between 15 and 30 times the square root of the additive variance . . .". Such a response cannot be explained by a small number of segregating loci. It has also been found that there is no appreciable change in the variance in the first few generations of selection. This favours the hypothesis of a large number of loci rather than that of a small number of loci.

There is, however, other evidence

which indicates that if the polygenic hypothesis did not exist it would have to be invented. Winter⁶, for instance, selected from commercial varieties of corn, two diverging lines for high and low protein and two lines for high and low oil content. After years of selection experiments, the difference between the high and low lines for oil content was more than 20 times the original standard deviation. Discussing these results, Student⁷ concluded that they could not be explained "on the basis of a few easily detected genes". He estimated the number at 100-300. Noting that there was no evidence that the selection had reached its limits, he concluded "that the number of genes affecting oil (or protein) content . . . may run up to thousands".

Fisher⁴ was critical of Student's⁷ estimate but felt that, in conjunction with other evidence, the results "... force one to the conclusion that all commercial varieties may be segregating in hundreds, and quite possibly, in thousands of factors influencing the normal development of a plant".

There seems to be no alternative hypothesis to account for these facts. Student^{7,8} did suggest an hypothesis to explain Winter's⁶ experiment, namely, that "species tend to accumulate a sufficient store of genes of no particular value until they meet with a change in environment when the store provides material for selection far beyond the normal range". This in no way diminishes the importance of the polygenic hypothesis; on the contrary, it strengthens it.

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of quantitative genetic traits, incorporating this information¹¹. This is not to say that the older facts are necessarily wrong, but rather that our perspective of the problem should be reconsidered.

Genes affecting continuously distributed phenotypes have small effects relative to uncontrolled environmental variation, and allelic substitutions at individual loci are essentially equivalent. This often makes precise genetic analysis difficult. It is clear from biometrical literature that models based on assumptions of many genes having similar phenotypic effects can explain quantitative variation quite elegantly. But are these assumptions generally valid? What experimental evidence is there to support the assumptions (that is, experimental evidence which is independent of the statistical tests, themselves)? Are there equally satisfactory models based on other reasonable assumptions about the number or magnitude of polygene effects?

An alternative assumption, to which Vetta objects, concerns polygene number. Evidence comes from both theoretical and experimental sources and is summarised briefly in my original article¹¹. One important observation is that theoretical distributions essentially indistinguishable from normal phenotypic distributions can be produced by segregation at a few loci, or even at a single locus. Several such examples are discussed in detail by Thoday and Thompson¹². The conclusion is that there is no justification for automatically assuming that an apparently normal phenotypic distribution is caused by the segregation of a large number of genes. Experimental evidence confirms this¹¹.

Some, but by no means all, characters continue to respond to artificial selection for "20 to 30 generations". Vetta is quite correct, therefore, in pointing out that there are some traits for which it is likely that a comparatively large number of factors contributes to the variance. Indeed, I did not deny that this may often be true (ref. 11, p. 666). I suggested, however, that these are examples of highly complex characters (that is, they are the result of the interaction of a large number of contributing developmental processes) and that polygenic influences on each of these contributing processes would tend to be cumulative. Quantitative variation in body weight, for example, may be produced by polygenes affecting the rate of bone growth or the adult bone size, the efficiency of nutrient uptake from the intestine, endocrine function, the rate of cell division, cell number and size, the size of muscles, and numerous other contributing processes which, if looked at

carefully, could each be considered quantitative characters in their own right. It is the analysis of these contributing processes which will give us the most rewarding insight into polygene number and function.

The point of contest should, therefore, not be whether there are "few" or "many" (at best, very unspecific terms), but whether the number of polygenes affecting any character is exceptionally large (the "hundreds" and "thousands" sometimes quoted). I believe that the balance of evidence provides no compelling reason to think that the number of polygenes affecting any particular trait is exceptionally large, and indeed, it is probably quite a lot smaller than generally thought.

The usual impression of multigenic effects is probably attributable, in many instances, to an unsophisticated approach to phenotypes and the processes of development. What is needed is an experimental approach to quantitative genetic variation which concentrates, not on the sheer number of factors¹, but on their functions and influences on development? The work by Spickett^{7,8}, Milkman¹³ and others shows that this is potentially more informative, for often the number of genes involved is not particularly large, and in appropriate conditions they can be isolated and manipulated readily⁹.

As long as continuously distributed phenotypes are regarded as necessarily the product of segregation at a large number of loci, straightforward chromosome substitution and other direct manipulations of the genome may not be given sufficient attention as possible alternatives to the more time-consuming and less precise techniques generally available to applied geneticists.

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THOMPSON REPLIES—Although I appreciate the position taken by Vetta¹ in defending the common hypothesis that quantitative variation is produced by the segregation of a large number of genes, much work has been done in the 30 or 40 years since Fisher, Winter, and 'Student' first considered the problem (see refs in Vetta¹). Among the most significant recent contributions to this problem have been those by Thoday and his colleagues²⁻⁴, particularly Spickett⁵⁻⁸, and those by Scharloo⁹ and Rendel¹⁰. It is well past time to reconsider our understanding

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reviews

Competition and community

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Ecology and Evolution of Communities. Edited by Martin L. Cody and Jared M. Diamond. Pp. xii+545 (Belknap for Harvard University: Cambridge, Massachusetts and London, 1975). \$17.70.

THIS collection of 18 papers which grew out of a memorial symposium by former students and associates of the late Robert MacArthur contains a brief summary of his work and a bibliography of his papers. Continuing in the MacArthur tradition, the contributors look for and explain the broad patterns of community organisation, often at the expense of detail. A major theme running throughout is the impact of competition on community structure.

In the longest paper of the symposium Jared Diamond explains alternate stable communities of birds on islands near New Guinea by combining information about dispersal and competitive ability. Martin Cody investigates the diversity patterns of bird communities in Mediterranean climates and Henry Hespdenheide assesses the importance of diet overlap in birds to species-packing problems. Using canonical correlation, James Karr and Frances James break new ground in identifying morphological and ecological correlates of bird communities. The negative correlation of bird species numbers with environmental variability suggests to John W. MacArthur that variability reflects the environmental resistance to invasion of new species. Taken together these studies provide strong support for the contention that competition is the major organising force in bird communities.

In papers dealing with non-avian communities—lizards (Eric Pianka), rodents (James Brown), butterflies (Arthur Shapiro) and diatoms (Ruth Patrick)—MacArthur's theories of species diversity seem less successful in explaining community structure. None of these papers directly confronts the basic premise that competition is the major kind of interspecific interaction. In general, predicted correlations match with the data but there are occasional bothersome discrepancies that either resist explanation or

require elaborate qualifications to the theory. The pre-eminence of competition is challenged by Joseph Connell in a very provocative essay in which he argues that predation is much more important, except in harsh physical conditions. Connell proposes that experimental manipulations of communities yield more information than comparison of unperturbed communities. In another paper in this volume, Richard Levins shows, however, that the unexpected effects resulting from perturbations of complex communities can be difficult to interpret.

There are also several major theoretical contributions. Michael Rosenzweig develops several models which predict an asymptote to species diversity on continents. Henry Horn proposes a model of forest succession as a Markovian process which he partially tests with data from the Princeton woods. He also manages to include a few enjoyable barbs at critics of this kind of general approach to succession. William Schaffer and Madhav Gadgil develop models to explain life history strategies for plants, and Leigh develops some models to relate population fluctuations and species diversity. Robert May presents a major synthesis of the theoretical underpinnings of species abundance relations, and distinguishes the biological from the mathematical properties of such distributions. His work should facilitate the testing of hypotheses in this area. In a difficult but very important paper, Levins outlines loop analysis, a tech-

nique enabling the analysis of communities where the direction but not the magnitude of the effect of one species on another is known. He uses loop diagrams to predict some unexpected consequences (such as a species selecting itself to extinction) of evolution in complex communities. The two concluding papers look ahead—G. Evelyn Hutchinson reviews the relationship between theory and data in several areas of ecology, and Edward O. Wilson and Edwin Willis point out some possible applications of MacArthurian theory to the preservation of diversity in natural areas.

In general the symposium participants share a common view of ecology—one with an emphasis on rather imprecise models that predict overall patterns. In this volume the major weakness emerging from this approach lies in the too often sporadic and incomplete statistical testing of theory. That some authors seemed too committed to competition theory when considering alternative hypotheses led to its occasional reification. With the exception of the contributions of Levins and Hutchinson, there was no clear sense of the direction in which ecological theory should develop. This volume is a good summary, however, of the status of that part of ecology strongly influenced by MacArthur and his associates. □

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Monumental work revised

The History of the British Flora: A Factual Basis for Phytogeography. Second Edition. By Sir Harry Godwin. Pp. x+541+12 plates. (Cambridge University: Cambridge and London, 1975.) £30; \$85.

THE publication of the second edition of Sir Harry Godwin's *History of the British Flora* has been an eagerly anticipated event in palaeoecological circles. There are few books which have had such a profound influence on the development of any branch of science than the first edition of this

book. The volume of additional information which has accumulated since its publication in 1956 is, in large measure, a singular monument to its success. As a stand of reeds accumulates new material around itself, ultimately resulting in its own replacement, so has Godwin's work provided a basis for the research of many students, thereby encouraging the accretion of new records and findings. The appearance of a second edition of this book can therefore be regarded as the inevitable outcome of an autogenic process.

By far the most important revision of the book is the incorporation of the newly assembled data into the encyclopaedic list of Quaternary fossil records of each taxon. This section is preceded by a list of the sites and references to publications from which information has been gleaned—a list in which one can detect a degree of selectivity in operation. Space is used more economically than in the first edition, for no longer are the locations listed for all occurrences of the commoner fossils. Instead records are represented as frequency histograms in the various subdivisions of the Quaternary. Godwin retains his standard I–VIII zonation system for the Late-Devensian and Flandrian and it is difficult to see how else he could provide a systematic framework for the arrangement of plant records. A full discussion of the radiocarbon dates associated with these zones, however, would have proved most valuable. It is now known that many of the changes in pollen assemblages which are used as a basis for the Godwin zonation system are not synchronous (with the possible exception of the zone VIIa/VIIb boundary) across



the country. But since the system has been widely used in the zonation of published pollen diagrams up to the time of the preparation of this book, it does provide the most obvious and widely understood method of presenting the data.

Godwin rightly claims that the fossil history of our flora provides a factual basis for the understanding of plant distribution patterns. It is also true that developments since 1956 have gone some of the way to-

wards the unravelling of phytogeographical problems. The science is, nevertheless, still in its infancy and the scope for new ideas and methods is immense. New techniques are now being brought to bear on palaeoecological studies, many of which have been borrowed from related disciplines; these could provide new insights as well as new information concerning the history of the British Flora. Meanwhile this book presents a full and authoritative account of the present position of this subject.

If this edition is indeed the product of a successional process, it should not be regarded as the climax. Although the body of information contained within the book is impressive, it should be looked on as a progress report rather than as a concluding statement. The reeds are growing taller and the ground is almost firm enough to walk on, but the process of accretion will continue and may soon result in some profound and unexpected developments. Production rates are such that we may need a third edition before another twenty years have elapsed and I, for one, look forward to it.

Peter D. Moore

Valuable source of limnological data

A Treatise on Limnology. Vol. 3: Limnological Botany. By G. Evelyn Hutchinson. Pp x+660. (Wiley-Interscience: New York and London, February 1976.) \$33.30; £16.75.

In his preface Hutchinson promises the remaining limnological data he has assembled in smaller segments. Yet this third volume on limnological botany encompasses only chapters 27–32 and is 660 pages packed with information, but virtually excluding work on rivers. As readers of the previous two volumes will know his scholarship is impressive and his search of the literature most thorough from sixteenth century herbals of Dodoens to publications of 1974. As usual an index of lakes and of genera and species is included.

The first 70 pages are devoted to the rooted cryptogams which in lakes comprise only the Charophyceae and Bryophyta. These are relatively neglected components of the aquatic flora and the author has had to quote extensively from a few major works. Nevertheless he has highlighted several relatively unstudied aspects and problems—for example, the confused taxonomic situation in the Charophyceae, the need for greater ecological data on occur-

rence, zonation, germination of oospores, and so on. In passing, the odours produced by organic compounds in *Chara* and the larvicidal effects of Charophytes on mosquitoes are brought to our attention. The relationship between distribution in different water types and depth zonation of both Charophytes and Bryophytes is dealt with; but as Hutchinson comments on p. 63 most published records say little about ecology, and experimental studies especially of aquatic Bryophytes are scarce.

The next 438 pages are devoted to aquatic tracheophytes, a few of which are Pteridophytes *sensu lato*; but most are Angiosperms, of which 51 genera of Dicotyledones and 65 Monocotyledones occur in water. In the second chapter these are figured, and their distribution and life forms described. Although interesting, much of this purely botanical information will be of slight interest to limnologists—particularly the numerous categories of life forms culminating in a seven-page table. The third chapter concerning the biological characteristics of aquatic plants is a mine of information, including photosynthesis, carbon sources, heterophylly, development of aerenchyma (including figures of an Eocene aquatic fern) seed germination, pollination and dispersal (including that of the well known *Elodea*).

The fourth chapter concerns the up-

take of nutrients, the chemical composition of the plant tissue and the distribution of the plants in relation to the chemical composition of the water. A large amount of data is assembled in this chapter, which forms the most comprehensive survey to date. The data on many chemical elements in water will be of interest, for example, to students of pollution. There then follows a chapter devoted to the depth distribution of macrophytes, the macrophytic flora of lakes selected from many regions, and a summary of the associations found in lakes based on phytosociological studies.

Finally the algal benthos in the widest sense (of all the algae, and briefly the lichens) associated with underwater surfaces) is summarised in a more adequate manner than in any other text, although here there is still a lack of data due to neglect of these communities in favour of the more easily studied plankton. The number of genera involved is very large and Hutchinson has not been able to survey these as comprehensively as those of the other freshwater associations.

There are only a few misprints, especially of authors names and some cross indexing without the page numbers. But otherwise this book is produced to the same high standard as the preceding volumes and alongside these it will remain an invaluable source for many years.

F. E. Round

Troubled waters

The North Sea: Challenge and Opportunity. (Report produced by Study Group of David Davies Memorial Institute of International Studies.) Pp. xiii+324. (Europa: London, 1975.) £6.95; \$21.00.

SINCE the discovery of hydrocarbons at exploitable depths in the British sector of the North Sea, public policy towards the area has been dominated by considerations of energy and little else. On land, considerations of ecology and the environment have acquired sufficient political force for the formation of a single Department of the Environment. The concept of land-use planning has become part of the vocabulary of bureaucrats, politicians, and the media elite. The same cannot, alas, be said for the sea, especially for the North Sea.

Although voices have been raised—on the National Environmental Research Council (NERC), in the recent Fabian Tract on Sea Use Planning, at the various conferences organised by the Greenwich Forum, and in various submissions to parliamentary committees—little has changed. To quote from the above report:

"... different activities in the North Sea are regulated at different levels and with varying degrees of thoroughness... It is only where a problem or situation has been seen as critical that measures have been taken to try to meet it. Otherwise inertia and the usual short-term view that characterises most politics prevails. Machinery is thus lacking for considering, evaluating and regulating for the long-term the complex of inter-related activities (which actually take place in the North Sea)... and this lack must be attributed to the absence of a general awareness of the need for such an integrated approach".

The report comments on the existence of a "widespread, if rather unfocused, realisation that there is a need for overall environmental planning. The danger, as always, is that lip service to an idea may become a substitute for hard thinking and eventual action; and this can postpone the recognition of the immediacy and gravity of the problem."

One of the most serious obstacles to such recognition in the past has been that those who recognise the "immediacy and gravity of the problem" in their own field are either scientists or technologists, lacking either the political understanding or the grasp of international issues necessary to convey their sense of urgency across those barriers to understanding or communication which divide the bulk of politicians, civil servants and leaders of opinion from the research laboratories, common rooms, technological seminars and institutes of engineering. The civil

servant or politician, however, has as much trouble bringing home to the numerate the compulsions of international law, the resistance of vested interests, the difficulties of reaching a democratic consensus, and the nature of political realities.

One of the many merits of this study group is that it effectively married expertise in environmental science and in international law. The chapters on the international and national aspects of the present legal regime in the North Sea should be essential reading for all concerned with the scientific and technological sides of the exploration, exploitation and development of the resources of the North Sea. Equally compulsory reading for all specialists should be the chapter on the competing uses and interests in the North Sea.

The report points to various obvious *lacunae* in our understanding of the North Sea: water budget; history of sedimentation; sea-air interaction; food chains; and basic chemical information on estuaries. To control man-made changes in the ecology of the North Sea very much more research needs to

be undertaken.

Its recommendations on the political level are, to my knowledge, streets ahead of the thinking of any government policymaking body in Western Europe, calling at the national level for a considerable reorganisation of national administration, involving the establishment of a UK Cabinet Committee with responsibility for inter-departmental collaboration and of a permanent parliamentary committee on the marine environment; and on the international level for the establishment of a regional regime for the North Sea in the form of a Standing Conference of North Sea States with an international economic committee and a joint North Sea policing service. It will alas be some time before these become politically feasible. What we need now is a survey of the policies of the other North Sea states to match that dealing with Britain.

This book cannot be too strongly recommended, at a time when so much of Britain's future depends on the way in which the opportunities provided by the recent discoveries in the North Sea are realised.

D. C. Watt

Student physics

Introduction to Modern Theoretical Physics, by Edward G. Harris. Vol. 1: Classical Physics and Relativity. Pp. xvii+383+9. Vol. 2: Quantum Theory and Statistical Physics. Pp. xvii+385-780+9. (Wiley-Interscience: New York and London, December 1975.) £13.50; \$27.00 each volume.

PROFESSOR HARRIS remarks that the tendency to specialise has increased to such an extent that large gaps occur in the education of PhD candidates in physics. He has attempted to write a textbook which covers everything such a student should know. The course covers mechanics, continuum mechanics, electromagnetism, relativity, general relativity, quantum mechanics, quantum field theory, elementary particles, thermodynamics, statistical mechanics and plasma physics.

The section on continuum mechanics is extremely short, whereas the treatment of general relativity is too ambitious (it includes attempts at unified field theory and geometrodynamics). The treatment of experimental questions in general relativity reflects the author's own enthusiasm, and in most subjects there is a balanced choice of topics. The author even has time for a few historical remarks. Much of the book overlaps, intentionally, with undergraduate

topics, even down to some things covered in the first year.

Although the intentions of the book are good, I doubt that it will ever become the English *Handbuch der Physik*. The section on classical mechanics is less good than H. Goldstein's book; the quantum mechanics section would not be preferred to Dicke and Wittke; and so on—each topic is already well covered by existing texts at the same level.

The use of imaginary time $x_4=ict$ instead of the usual $x_0=ct$, leads to confusion between hyperbolic and elliptic equations. The author is over-cautious in condemning the Klein-Gordon equation as having "unsatisfactory features". The treatment of boundary conditions on the Schrodinger wave function is left vague with the statement that ψ should be "well-behaved". Understanding this point is not helped by his advocacy of the Dirac bra and ket formalism. A simple explanation is found in Kemble's book—one requires self-adjoint boundary conditions. The author is also clearly out of his depth (p. 400) when mentioning unbounded operators. I would have preferred the theory of the Dirac sea to have been left unsaid; its inconsistencies were bypassed by second quantisation 45 years ago.

Nevertheless, a physicist who knows everything in these books will be widely educated in modern theoretical physics.

R. F. Streater

Astronomy: The Evolving Universe. By Michael Zeilik. Pp. xii+529. (Harper and Row: New York and London, 1976.) n.p.

This book will be jostling with about a dozen superficially similar texts for a slice of the American college market, since it is an integrated course aimed at students with an interest in astronomy. What differentiates it from its competitors are two features: it is up-to-date (about 1975), and it weaves the essential science around an intriguing theme, the evolution of the universe and the objects within it. The classical ordering of material is fast falling into disuse as authors seek cosmetic devices to disguise the essential similarity of their books. This one certainly wears an attractive face.

Zeilik's text spreads itself at great length across four major topics: man's changing ideas about the universe; the solar system; stars and galaxies; and cosmic evolution. The test particles so dear to reviewers (black holes, pulsars, exobiology, nucleosynthesis, and quasars) are all here and explained with admirable clarity, along with massive doses of astronomical history, planetary science, and astrophysics.

I believe that this is a really outstanding teaching text for qualitative courses. Every page is packed with facts, and the error rate is astonishingly low. Zeilik clearly understands what writing for students is all about: his material is clear, interesting, correct and complete. Among the many new things I learned was that reading books printed entirely in royal-blue ink is very tiring. Otherwise this is a fine effort that is certain to be adopted for general courses in the US and Canada.

Simon Mitton

Principles of Enzyme Kinetics. By Athel Cornish-Bowden. Pp. 206. (Butterworths: London and Boston, Massachusetts, February 1976.) £12.

THE author of this work set himself the task of covering the major aspects of enzyme kinetics in a relatively small book, and the result of this extremely ambitious attempt is that the coverage of the different topics is rather uneven. At best the book is very good indeed and the accounts of the principles behind the schematic methods for deriving kinetic equations and the analysis of progress curves are clearly and logically presented. Some of the other sections are, however, less satisfactory. In the chapters on fast reactions and on the effects of pH and temperature, for example, the author tends to select aspects of the subjects for short com-

ments without providing any detailed account of the general principles involved in the analysis and interpretation of experimental results. In addition the condensed nature of the book leads to the author making a number of categorical statements without any justification, some of which might be regarded as being arguable by others working in the field. For anyone with a reasonable knowledge of enzyme kinetics this book should offer a number of interesting insights, but the patchy coverage does not recommend it as an introductory textbook for the subject.

Books brief

In spite of its limitations I found the book to be extremely valuable for its excellent treatment of statistical methods for evaluating kinetic results. This is a subject that has received scant attention in the past and the lucid account presented here should be of great help to anyone attempting to interpret the results of kinetic experiments. **Keith Tipton**

Antimetabolites of Nucleic Acid Metabolism: The Biochemical Basis of Their Action, with Special Reference to Their Application in Cancer Therapy. By Peter Langen. Pp. xii+273. (Gordon and Breach: New York, London and Paris, December 1975.) £10.

THIS is a rather dull, very condensed and encyclopaedic account of the nature and mode of action of a wide range of anti-metabolites affecting the biosynthesis and function of nucleic acids. It is not a readable book, partly because of the prose style, but also because of the type face used and the somewhat inadequate legends to many of the illustrations.

The first five chapters deal with historical and general features of anti-metabolites, mode of action, acquired resistance, degradation and biochemical aspects of cancer chemotherapy. Each chapter opens with some general considerations before proceeding to illustrate these with specific and detailed reference to particular compounds.

The second part of the book tends to be something of a catalogue of anti-metabolites, some of which receive substantial treatment, whereas

others are dealt with very briefly indeed.

In the foreword, the authors indicate that this new edition contains much new material, but it is a pity that some of this has been dealt with in an addendum rather than being integrated into the text.

As it stands, the book contains a great deal of information and a very extensive bibliography. It will certainly be of value to research workers in this field by drawing together information and ideas from widely scattered sources, but it is a book for a reference library rather than for the individual purchaser. It is unfortunate that it does not include a detailed alphabetical index.

R. M. S. Smellie

Organic Reaction Mechanisms—1974. (An annual survey.) Edited by A. R. Butler and M. J. Perkins. Pp. 659. (Wiley-Interscience: London, New York, Sydney and Toronto, February 1976.) £25.00; \$55.00.

THIS volume, produced by two new editors, follows closely on the pattern set by its predecessors—in itself an excellent recommendation. The period covered is December 1973 to November 1974 and being the tenth volume in the series it incorporates the second quinquennial index. The subdivision of the subject matter has not been changed substantially—reactions of aldehydes and ketones and their derivatives, including enzymic reactions (B. Capon), reactions of acids and their derivatives again including sections of enzymic catalysis and reactions (M. I. Page), radical reactions (D. C. Nonhebel and J. C. Walton), oxidation and reduction (T. W. Bentley), carbenes and nitrenes (M. S. Baird), nucleophilic aromatic substitution including sections on Meisenheimer complexes and benzyne (M. R. Crampton), electrophilic aromatic substitution (B. V. Smith), carbonium ions (R. Baker), nucleophilic aliphatic substitution (I. D. R. Stevens), carbanions and electrophilic-aliphatic substitution (R. B. Boar), elimination reactions, I, polar addition (A. C. Kripe), addition reactions, II, cyclo-addition (W. E. Watts) and molecular rearrangements (A. R. Butler, G. V. Meehan and M. J. Perkins). Each chapter is clearly subdivided, amply illustrated by formulae, fully referenced, and the overall format is of a high standard. The price of the volume is very high even allowing for the detailed content, but this series is a must for all well-stocked libraries of organic chemistry. **A. W. Johnson**